Performance of hybrid subsurface flow constructed wetland system used for high content wastewater treatment

（高濃度排水の浄化処理を行うハイブリッド伏流式人工湿地システムの性能に関する研究）

Hokkaido University　　Graduate School of Agriculture
Division of Environmental Resource　　Doctor Course

Xiaomeng Zhang

Content

Figures

Tables

Summary

Hybrid subsurface flow constructed wetlands (CWs) that consist of vertical flow (VF) CWs and horizontal flow (HF) CWs have being used world wide to treat various kind of wastewater, with the advantages of low cost, low energy consumption, low maintenance requirement, and environmental friendliness. High content wastewater (with chemical oxygen demand (COD) of 3,752 ± 2,071 mg•L^{-1} to 10,961 ± 3,146 mg•L^{-1}. that discharged from piggery farm and dairy farm without efficiency treatment could cause environmental problems such as surface water and groundwater pollution, water eutrophication, and bad odors.

With the purpose of treating high content wastewater, three hybrid CWs were constructed to treat drainages from a piggery farm and two dairy farms. The objectives of this study is to evaluate pollutant treatment performance of these three hybrid subsurface CWs over several years of operation, and to better uunderstand the N balance and transformations in hybrid CWs.

Bimonthly, water samples were collected at the inflow of each bed and final outflow from the beginning of hybrid system's operation. Bed material samples were collected for N balance analysis in June 2014 and August 2014.

After 6 years, 4 years and 9 years of operation for Piggery-O, Dairy-G and Dairy-S, respectively, the performances of whole systems were assessed.

Totally, the purification efficiencies of T-N in these three systems were 72 ± 9% ~ 86 ± 12% in these hybrid systems, which indicated these systems performed well for N

removal. The systems also presented high COD and BOD_5 purification efficiencies of around 94 ± 5% to 96 ± 2% and 94 ± 4% to 98 ± 4%, while they received high content of COD and BOD_5.

In Piggery-O, Dairy-G, and Dairy-S, the influent T-N loads were 11.2 ± 7.5, 3.4 ± 1.7, and 1.0 ± 0.5 g•m^{-2}•d^{-1}, respectively. Overall T-N removal efficiencies were 71 ± 20%, 81 ± 9%, and 82 ± 15 % in these hybrid systems. NH_4-N removal rate were 7.1 ± 5.3, -0.2 ± 0.2, and 0.4 ± 0.2 g•m^{-2}•d^{-1} in Piggery-O, Dairy-G, and Dairy-S, respectively.

Piggery-O, Dairy-G, and Dairy-S showed T-P removal rates with amount of 1.0 ± 0.9, 0.5 ± 0.2, 0.1 ± 0.1 g•m^{-2}•d^{-1}, respectively. And T-P removal efficiencies were 90 ± 10%, 70 ± 11%, and 64 ± 29% for Piggery-O, Dairy-G, and Dairy-S, respectively.

COD removal rates were 49.7 ± 50.4, 118.4 ± 57.1, and 25.6 ± 15.3 g•m^{-2}•d^{-1} for Piggery-O, Dairy-G, and Dairy-S, respectively. Dairy-G had higher influent load of 124.0 ± 58.5 g•m^{-2}•d^{-1} COD compared with 27.6 ± 15.8 g•m^{-2}•d^{-1} of dairy S. Total COD removal efficiencies for Piggery-O, Dairy-G, and Dairy-S were 93 ± 10%, 95 ± 2%, and 93 ± 8%, respectively. Dairy-G also had highest removal rate of BOD_5, with amount of 84.9 ± 52.2 g•m^{-2}•d^{-1}.

After years of operation, Piggery-O presented an increasing T-N removal efficiency year by year, and provided stable and high T-P removal efficiency. Diary-G had high removal efficiency of T-N from the beginning of construction, and it was stable after years of operation. While T-P removal efficiency decreased from 80 ± 6% in the first year to 69 ± 11% in the forth year. Similar T-P removal tendency was also observed in Dairy-S, due to saturation of the bed material adsorption capacity over time.

All these hybrid systems had high and stable annual removal efficiencies of COD and BOD_5 since the beginning of construction. Overall, these hybrid CWs systems could be recognized as useful and effective methods for piggery and milking parlor wastewater treatment over years of operation.

After years of operation, the accumulated amount of T-N removed by Piggery-O was 15,579 kg, while the amount of T-N accumulated in bed material were 1,358 kg. These suggest that a 91% of N removed by this system was released into atmosphere in form of gas produced by denitrification. At each bed of Piggery-O, except that part of N loaded out to the next bed stage, only 1~2% of received N was stored in bed materials, while 8 ~ 37% of received N was converted into N gaseous, and then released into astrosphere. In Piggery-O, N balance analysis also indicated that the surface organic matter layer contained 36.4 % of T-N that stored in the system. Among the bed materials, ALC took account of 21.6 % T-N absorption, while the N stored in porous pumice gravel was 42.0 %. For Dairy-S, the whole system removed 1,795 kg, T-N, while the amount of T-N stored in beds was 869 kg, and nearly 52% of received N was released into atmosphere. In addition to large amount of received N was converted into N gaseous, nearly 9~35 % of received N stored in each bed materials. In the 1^{st} bed, where nearly 690 kg of N was stored in the surface covering organic matter layer, which contained 87.3 % of T-N removed by system bed material. This indicated that surface organic matter layer might be an un-negligible way for N removal in hybrid system treating milking parlor wastewater. Totally, bed materials such as pumice gravel, ALC as well as clinker ash had high N absorptivity compared with river gravel and sand.

Chapter 1 Introduction

1.1 Background of constructed wetland

1.1.1 Instruction for constructed wetland

Wastewater that discharged into watersheds or surrounding environment without treatment or efficiency treatment, would has serious impacts on environment quality and would pose a serious threat to aquatic life and pubic health. Further more, wastewater can negatively affect the use of water for drinking, household needs, recreation, fishing, transportation, and commerce (Doosti *et al*., 2012). For example, high content nitrogen into natural water would cause eutrophication of lakes and rivers (Xinshan *et al*., 2010), which results in poor water flavor and odor. Untreated organic materials often deplete dissolved oxygen (DO) concentration availability, leading to the death of aquatic organisms.

Many Conventional wastewater treatment processes are designed to achieve improvements in the quality of the wastewater, such as active sludge process (ASP), rotating biological contactor (RBC), stabilization ponds, and sequence batch reactors (SBR). Compared with other wastewater treatment methods, constructed wetlands (CWs) have the advantages of simple construction and low maintenance and eco-friendly (Cooper, 1999; Kadlec and Wallace, 2009; Saeed and Sun, 2012; Tanner and Sukias, 2003; Vymazal, 2007).

Take advantage of processes that occur in natural wetlands, constructed wetlands are designed and constructed by use of rooted water tolerant plants and gravel or soil media (Kadlec and Knight, 1996). Hence they could create more controlled ecological conditions for treating wastewater in physical, chemical and biological ways (Vymazal, 2005; Wallace and Knight, 2006). CWs that used for sewage, industrial, agricultural, landfill and others wastewater treatment have been reported in recently decades (Cooper, 1999, 2001; Gaboutloeloe *et al*., 2009; Vymazal, 2005, 2013).

There are several categories of CWs according to macrophytes and water flows. Based on the type of macrophytes, CWs are classified into 4 groups: free-floating macrophytes, floating-leaved macrophytes, submerged macrophytes and emergent macrophytes. Depending on wastewater flow levels, CWs could be named as free water surface (FWS) and subsurface flow (SSF). Surface flow CWs are similar to natural wetlands, with shallow flow of wastewater (usually less than 60 cm deep) over saturated substrate. Subsurface flow CWs usually use gravel, sand, or other filters as the main media to support the growth of plants. Also, according to wastewater flow pattern that pass trough media matrix of CW, subsurface flow CWs could be divided into horizontal flow (HF) and vertical flow (VF) (Vymazal, 2008; Imfeld *et al*., 2009). When wastewater flows vertically or horizontally through the substrate, it begin to contact with microorganisms living on the surfaces of plant roots and substrate, and pollutants were removed from the wastewater (Cooper *et al*., 1996; Kadlec and Knight, 1996). In addition, based on the flow direction, VF CWs have up flow and down flow types.

Hybrid CWs that consist of various types of CWs could cover the limitation of each single CW, and achieve higher treatment effect. And in hybrid subsurface flow CWs, mainly HF and VF CWs are used to complement each other to provide suitable conditions for nitrification and denitrificaton. VF CWs can provide a good condition for nitrification due to aerobic conditions existed in the filter bed. HF CWs are often in anaerobic conditions due to the limited oxygen transfer capacity of the bed, and hence provide conditions for denitrification (Vymazal, 2005, 2007). It was also documented that VF CWs are extremely effective in removing suspended solids and BOD_5 (Brix *et al.*, 2002).

Treatment for wastewater from Piggery and dairy farm

Livestock is one of the main sources of point source nitrogen contamination. According to global level, livestock industry released 135 million tones of nitrogen in the form of excreta and Asia is the largest contributor (Food and Agriculture Organization of the United Nations). In recent decades, there have been a number of studies regarding high content wastewater, such as piggery urine and dairy parlor wastewater discharge. Cronk (1996) studied CW treatment of wastewater from dairy and swine operations and found that surface flow wetlands were most common. Shamir *et al.* (2001) used surface flow CWs to treat dairy wastewater. Kantawanichkul and Somprasert (2005) studied the efficiency of a pig farm wastewater treatment CW, featuring VF above HF. There have also been studies regarding CW performance for treatment of livestock and dairy farms' wastewater (Borin *et al.*, 2013; Dunne *et al.*, 2005; Healy *et al.*, 2007; Knight *et al.*, 2000; Reeb and Werckmann, 2005; Tanner *et al.*, 2005). Among the studied high content wastewater treatment systems, most of them were not hybrid subsurface flow CWs. Some hybrid systems have been evaluated, but many of those studied have been in a pilot phase or built to an experimental scale. There is limited data regarding field scale hybrid subsurface flow CW systems.

As in Japan, livestock industry generates about 83 million ton of waste on an annual basis (28% from dairy; 29% from beef cattle; 27% from swine; 9% from layer and 6% from broiler) (Ministry of Agriculture, Forestry, and Fisheries, Japan), and roughly 25% of all the complaints filed against livestock industry are related to water pollution (Ministry of Agriculture, Forestry, and Fisheries, Japan). Discharge standards (total nitrogen limit of 120 N-mg /L, daily average of 60 mg-N/L) have been applied to facilities that are releasing more than 50 m^3 per day. As of 2014 and 2015, there were 246 piggery farms and 6,680 dairy farms in Hokkaido, Japan (Ministry of Agriculture, Forestry, and Fisheries, Japan). These farms produce large amount of high content wastewater, which must be effectively treated to prevent environmental problems.

However, although CWs are convenient for wastewater treatment, clogging problems might occur in CWs after years of operation, because pollutants accumulated in the CWs could result in gradual clogging of the medium (Cooper *et al*., 2008; Nivala *et al*., 2012). The longevity of CWs in the literature varied in recently years, from 15 years (Bavo and Schulz, 1993) to 10 years (Wallace and Knight, 2006), and to 8 years recently (Griffin *et al*., 2008). So the long-term assessment of CW performance should be preceded. Moreover, there are lesser data regarding long-term hybrid subsurface flow CW performance.

1.1.2 Removal mechanism

Hybrid CWs provide ideal environment for wastewater treatment. Different types of pollutants could be removed through a complex cooperation of plants, media, bulk water and biomass population (Fountoulakis *et al.*, 2009).

N removal

Nitrogen exists in wastewater mainly in inorganic and organic forms. The inorganic nitrogen including ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), nitrogen gas (N_2), nitrous oxide (N_2O), nitric oxide (NO_2), nitric oxide (NO), and free ammonia (NH_3). Among the gaseous nitrogen, N_2O is a kind of green house gas. The organic nitrogen is usually in form of amino acid, urea, uric acid, purine and pyrimidine (Kadlec and Knight, 1996). If the raw wastewater were rich with organic nitrogen, ammonification would occur firstly to transform organic nitrogen into ammonium (Savant and DeDatta, 1982).

Nitrification

If NH_4-N is dominant in wastewater, the step of Nitrification was presented. NH_4-N is nitrified into nitrite (NO_2-N) then into nitrate (NO_3-N) with the help of chemolithotrophic bacteria. The overall nitrification reaction in Kadlec and Knight' s report (1996) was written as:

$$NH_4^+ + 1.83\,O_2 + 0.98HCO_3^- \rightarrow 0.021\,C_2H_7NO_2 + 1.04H_2O + 0.98\,NO_3^- + 1.88H_2CO_3$$

Equation 1-1

Denitrification

If the organic compounds presented in wastewater and serve as electron donors, denitrification occurs. NO_3-N is transformed into N_2, N_2O or NO. In this step, facultative bacterial groups were involved. The denitrification reaction is as follows (Kadlec and Knight, 1996):

$$NO_3^- + 1.08\,CH_3OH + 0.24H_2CO_3 \rightarrow 0.056\,C_5H_7NO_2 + 1.68H_2O + HCO_3^- + 0.47N_2$$

Equation 1-2

Besides these classical N removal routes (ammonification, nitrification, denitrification, plant uptake, biomass assimilation, dissimilatory nitrate reduction, ammonia volatilization, and adsorption), new discovered N removal rates processes were reported: such as partial nitrification-denitrification (Jianlong and Ning, 2004; Zhang *et al.*, 2011), Anammox (Anaerobic ammonium oxidation) (Strous *et al.*, 1997) and Canon (Completely autotrophic nitrogen removal over nitrite) (Third *et al.*, 2001).

Organic compounds

Organic matter could be decomposed by both aerobic and anaerobic microbial processes, as well as by sedimentation and filtration of particulate organic matter in CWs. Organic matter is composed of a complex mixture of biopolymers (Megonikal *et al.*, 2004). For soluble organic matters, aerobic degradation is preceded by the aerobic heterotrophic bacteria. These bacteria use oxygen as the final electron acceptor (Garcia *et al.*, 2010), and could oxidize organics into carbon dioxide. If the system were lack of oxygen, the performance of aerobic biochemical oxidation would be reduced. The process of aerobic degradation is as follows:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6CO_2 + 6H_2O \qquad \text{Equation 1-3}$$

For anaerobic degradation, they were carried out by anaerobic hetertrophic bacteria, the organic compounds were converted into new bacterial cells, methane and carbon dioxide through several steps, for example, fermentation and methanogenesis occur in anaerobic zones of wetlands (Kadlec and Knight, 1996).

Phosphorus

Phosphorus (P) presented in wastewater primarily as phosphate in organic and inorganic compounds (Vymazal, 2007). Organic P forms include easily decomposable P (nucleic acids, phospholipids or sugar phosphates) and decomposable organic P (inositol phosphates or phytin) (Dunne and Reddy, 2005). P transformation in wetland includ peat/soil accretion, soil adsorption and precipitation, macrobiotic uptake, and plant uptake. Usually, microbial uptake and plant uptake of P in CWs is low. Depending on the types of CWs, the P transformation potential varies. When special filtration materials are used, the HF CWs and VF CWs performed high adsorption, and low precipitation occurs especially if gravel is used. Overall, P removal in CWs including sorption on substrates, storage in biomass and sediments (Kadlec and Knight, 1996). When CWs substrates become saturated with P, the removal capacity varies (Dunne and Reddy, 2005). When wastewater has low concentrations of P, there is a net movement of P from substrate to water until there is equilibrium between substrate and pore water

P concentrations. In addition, Phosphate can be released (desorbed) from the metal complexes depending on the redox potential of the sediment.

Suspend Solid

VF and HF CWs could provide opportunities for suspend solid (SS) separations by gravity sedimentation, adsorption on biomass film attached to gravel and root systems.

1.1.3 Factors that affect pollutant removal

In order to better understand hybrid subsurface flow CW performance, there have been some studies regarding the influence of environmental factors upon treatment efficiency. Environmental factors, such pH, dissolved oxygen (DO), temperature, and redox condition could affect the removal of N and organics in CWs.

Literature reports indicate that the highest rate of denitrification is observed at a pH range 7.0-7.5 (U.S. EPA, 1975, Saeed and Sun, 2012), and ammonium volatilization might presented while the pH rarely surpasses 9.0.

Considering denitrification, the DO concentration should be maintained at < 0.3-0.5 $mg•L^{-1}$, to accomplish nitrate reduction (Saeed and Sun, 2012), and organic compound would compete with nitrification if system were lack of DO (Tanner and Kadlec, 2003). Artificial aeration could significantly improve oxygenation for nitrification in hybrid subsurface flow CWs (Maltais-Landry *et al*., 2007).

The literature review reported that nitrification was dependent on temperature; the researchers found favorable conditions for nitrification between 16.5°C and 32°C, and unfavourable conditions at lower temperatures (Saeed and Sun, 2012). In summer period, the hybrid system had high removal efficiency of N and organics compared with in winter period (Tuncsiper, 2007; Langergraber *et al*., 2007; Nivala *et al*., 2007). In the study reported by Paredes *et al*. (2007), nitrite oxidation is inhibited and accumulated when the DO concentration is lower than approximately 2.5 mg•L^{-1}. Mietto *et al*. (2015) also reported that temperature affected N removal in a hybrid system.

There are a number of studies regarding the addition of carbon from external sources and the effect of C/N ratio on the treatment efficiency (Isasscs *et al*., 1995; Kim *et al*., 1997; Zhao *et al*., 2011; Zhu *et al*., 2014).

1.2 Objectives

This study focus on the treatment of CWs used for urine wastewater from a piggery farm with 159±60 to 1,433±342 mg total N (T-N) L^{-1}, and milking parlor wastewater from two dairy farms with chemical oxygen demand (COD) of 3,752±2,071 ~10,961±3,146 mg•L^{-1}.

This study built upon the previous research reported by Kato *et al*. (2013a,b) and Sharma *et al*. (2011, 2013). And this study could offer design and operational methodologies for further research.

The objectives of this study are: (1) to evaluate the performance of full-scaled hybrid constructed wetland treating high content piggery wastewater and milking parlor wastewater over years of operation in cold climate; (2) to evaluated environmental parameters, as they relate to pollutant removal efficiency; (3) to provide the N balance and transformations in CWs constructed with different filter materials.

1.3 Thesis outline

Chapters 2 mainly focus on materials and methods used in this study. Chapter 3 indicates the performance of hybrid systems, including the wastewater purification efficiency and removal efficiency of each system, pollutant removal efficiency at each bed of the whole system, system performance in both warm and cold period, and the yearly treatment tendency. Factors that affect pollutants treatment efficiencies were analyzed. Chapter 4 illustrated the N balance and transformations in Piggery-O and Dairy-S. Chapter 5 includes the conclusions of the whole study.

Chapter 2 Materials and methods

2.1 Study site

The hybrid subsurface flow CW system treating livestock wastewater from O piggery farm is located in Chitose (Piggery-O, N42º49′, E141º44′), the system treating milking parlor wastewater from G dairy farm is located in Takinoue (Dairy-G, N44º8′, E143º2′), and the system treating milking parlor wastewater from S dairy farm is located in Embetsu (Dairy-S, N44º45′, E141º48′), Hokkaido, Japan (Fig. 2-1). Dairy-S was located in a heavy snow falling area, and the annual total precipitation of Embetsu was 1,126 ± 195mm.

Basic meteorological information such as average air temperature, average precipitation, construction year and assessment period is shown in table 2-1. Assessment period in this study is from the beginning of system operation to December 2015. Average maximum air temperatures were 31 ± 2˚C, 34 ± 1˚C, and 30 ± 1˚C and average minimum air temperatures were -23 ± 1˚C, -30 ± 0.7˚C, and -21 ± 22˚C for Piggery-O, Dairy-G, and Dairy-S, respectively. Local meteorological data were provided by Automated Meteorological Data Acquisition System (AMeDAS) of Japan Meteorological Agency.

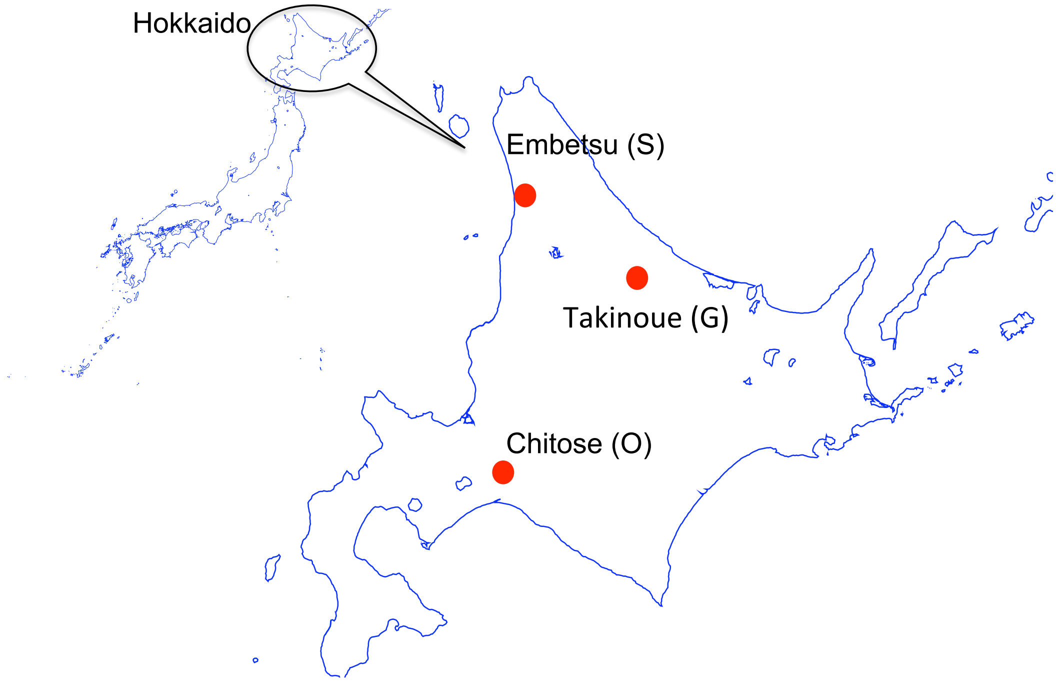

Fig. 2-1 Location of three hybrid subsurface flow CWs in Hokkaido, Japan.

Table 2-1 Precipitation, temperature, construction year of the hybrid subsurface flow CWs, and assessment period.

System	Location	Annual precipitation (mm)	Annual air temperature (°C)	Constructed year	Assessment period
Piggery-O	Chitose	1,018±83	7.4±0.4	2009	Dec.2009-Dec.2015
Dairy-G	Takinoue	1,060±114	7.1±0.3	2011	May 2011-Dec.2015
Dairy-S	Embetsu	1,126±195	7.0±0.4	2006	Nov.2006-Dec.2015

Note: Average values represent mean ± standard deviation.

2.2 Raw wastewater quality and characteristics

Piggery-O system received piggery urine wastewater from a pig farm, where there were 2000 pigs and cows. The average inflow rate was 11.6 ± 7.1 $m^3 \bullet d^{-1}$, with an original COD concentration of 6,340±3,396 $mg \bullet L^{-1}$, and T-N concentration of 1,433±342 $mg \bullet L^{-1}$. Dairy-G system received washing milking parlor wastewater from a dairy farm breeding 500 milking cows. The average inflow was 33.2 ± 6.9 $m^3 \bullet d^{-1}$. T-N original inflow concentration was 300 ± 92 $mg \bullet L^{-1}$, while COD influent concentration was 10,961 ±3,146 $mg \bullet L^{-1}$, which was highest among these three hybrid systems. For Dairy-S which was also used for milking parlor wastewater treatment, received an inflow of 4.8 ± 0.9 $m^3 \bullet d^{-1}$, and COD influent concentration was 3, 752 ± 2,071 $mg \bullet L^{-1}$, and for T-N inflow concentration was 159 ± 60 $mg \bullet L^{-1}$. There were 120 milking cows in the dairy farm. More details of original inflow characteristics of 3 hybrid systems were shown in Table 2-2.

Table 2-2 Wastewater original inflow concentrations of 3 hybrid systems.

	T-N mg•L^{-1}	NH_4-N mg•L^{-1}	T-P mg•L^{-1}	COD mg•L^{-1}	BOD_5 mg•L^{-1}	SS mg•L^{-1}	T-C mg•L^{-1}	PO_4-P mg•L^{-1}	Org-P mg•L^{-1}	Org-N mg•L^{-1}
Piggery-O	1,433 ±342	1,099 ±455	148 ±59	6,340 ±3,396	1,914 ±1,693	1,640 ±1,267	3,073 ±1,352	52 ±27	96 ±52	313 ±285
Dairy-G	300 ±92	9 ±4	60 ±18	10,961 ±3,146	7,093 ±3,076	2,816 ±897	3,126 ±550	40 ±10	11 ±6	291 ±92
Dairy-S	159 ±60	67 ±26	26 ±11	3,752 ±2,071	1,446 ±590	652 ±478	1,192 ±621	21 ±10	5 ±2	91 ±50

Note: Average values represent mean ± standard deviation

2.3 Hybrid subsurface flow constructed wetlands

2.3.1 Outlines of hybrid systems

2.3.1.1 Piggery-O

Schematic diagram of Piggery-O is shown in Fig.2-2. Piggery-O hybrid subsurface flow CW is consisted of four VF (V) beds and one HF (H) bed, in form of Vr-Vr-V-H-V, where Vr means wastewater recirculation occurs in the V beds. After piggery slurry discharged from the farm was separated into solid part and liquid part, the liquid part was dosed into the hybrid system. Part of the effluent from the 3rd V was pumped into 1st Vr and 2nd Vr in order to improve total performance. The surface of 1st Vr was divided into three zones while 2nd Vr and 3rd V were divided into two zones, these zones could be used alternately to maintain dry conditions like in a French system (Molle *et al.*, 2005). The resting and feeding periods were 1-2 weeks.

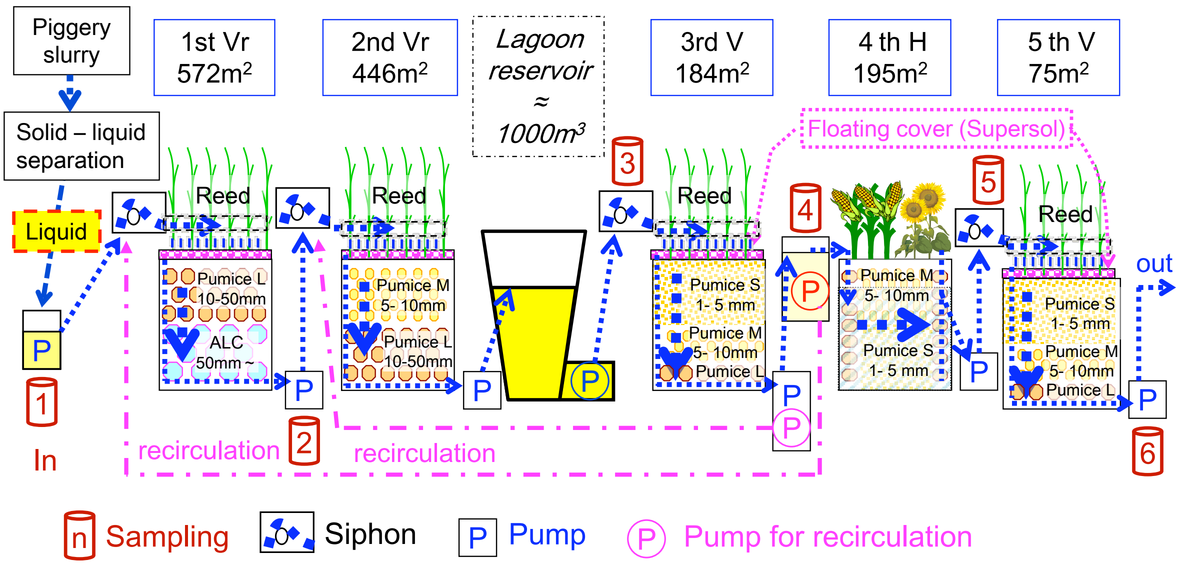

Fig. 2-2 Schematic diagram of Piggery-O hybrid system.

In order to avoid clogging and frozen problems that would be happen in hybrid systems treating dense wastewater in cold climates, several steps were taken (Kato *et al*., 2013a,b). A safety bypass structure (Fig. 2-3) was used as an "emergency door". When bed surface was temporarily clogged and percolation stopped, by using of this bypass, wastewater drained downward through these bypass pipes. In addition, a floating cover material named Supersol (TRIM Co., Ltd., Okinawa, Japan) was used to overcome clogging and frozen in cold climate, because that it could floats on the water and act as an obstruction to surface flow to change partially clogging and act as an insulating material to prevent frozen in winter.

Fig. 2-3 Surface partition and reinforced safety bypass.

Total area of Piggery-O was 1,472 m^2, volcanic porous pumice gravel with different size (L, large size; M, middle size; S, small size) was used as the main bed material. Construction details such as each bed area, bed materials, surface covering material, and main vegetation were shown in Table 2-3. Common reed (*Phragmites australis*) was the main vegetation planted in 1st Vr, 2nd Vr, 3rd V and 5th V, and it was not harvested. For 4th H, various upland crops and wetland flower species were planted to evaluate the adaptation of different plants.

Table 2-3 Bed type and area, depth, main bed material, surface covering material, and vegetation of Piggery-O.

System	Bed type	Bed area (m^2)	Depth (m)	Main bed material	Surface covering material	Main vegetation
Piggery-O	1st Vr	572	0.65	PG	ALC	Phragmites
	2nd Vr	446	0.65	PG	Supersol; ALC	Phragmites
	3rd V	184	0.65	PG	Supersol; ALC	Phragmites
	4th H	195	0.68	PG	Supersol; ALC	Upland crops
	5th V	75	0.65	PG	Supersol; ALC	Phragmites

Note: ALC: autoclaved lightweight aerated concrete. PG: Pumice gravel.

2.3.1.2 Dairy-G

As shown in Fig. 2-4, Dairy-G system is consisted of four vertical beds and a horizontal bed, in form of Vr-V-V-H-V. Part of the effluent from the 3rd V was pumped into 1st Vr. The surface of 1st Vr was divided into three zones while 2nd V and 3rd V were divided into two zones. Supersol was also used as main cover material in 1stVr to avoid clogging problems. In addition, bypass structure was also used in this system.

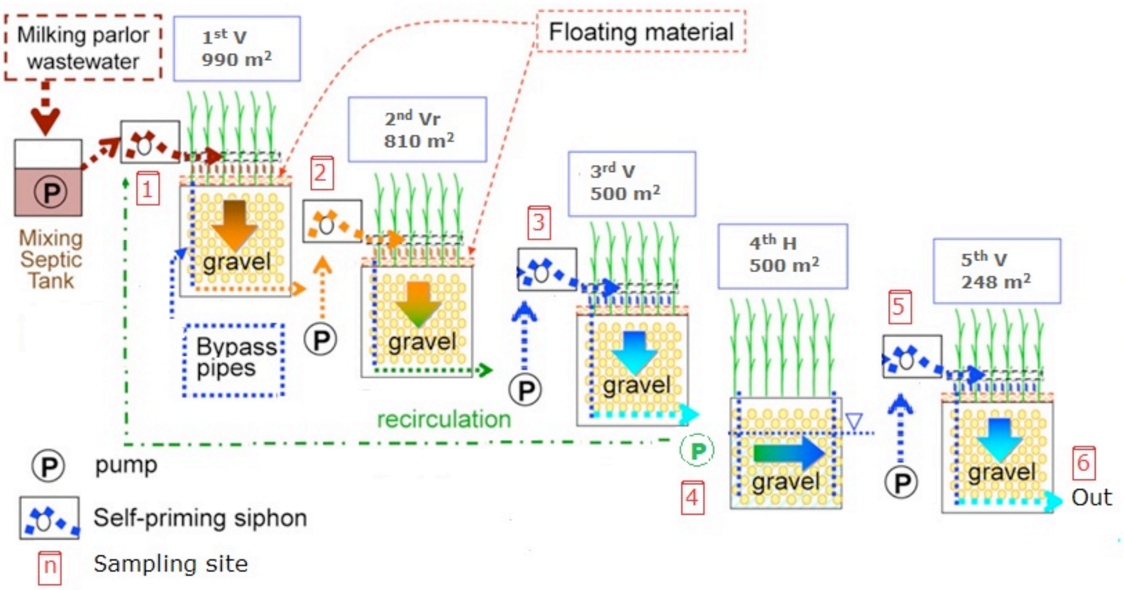

Fig. 2-4 Schematic diagram of Dairy-G hybrid system.

Total area of Dairy-G was 3,048 m^2, the bed depth was around 0.75m. River gravel was used as the main bed material in this system, and Supersol was the main surface covering material. More construction details were shown in Table 2-4. Common reed (*Phragmites australis*) was main vegetation planted in this system and it was not harvested.

Table 2-4 Bed type and area, depth, main bed and surface covering material, and vegetation of Dairy-G.

System	Bed type	Bed area (m^2)	Depth (m)	Main bed material	Surface covering material	Main vegetation
Dairy-G	1st Vr	990	0.75	River gravel	Supersol	Phragmites
	2nd V	810	0.75	River gravel	Supersol	Phragmites
	3rd V	500	0.70	River gravel	Supersol	Phragmites
	4th H	500	0.70	River gravel	Supersol	Phragmites; others
	5th V	248	0.65	River gravel	Supersol	Phragmites

2.3.1.3 Dairy-S

Fig. 2-5 shows the schematic diagram of Diary-S system treating milking parlor wastewater. Diary-S system is consisted of two V beds and one H bed, in form of V-Vr-V. Part of the effluent from the 2nd Vr was pumped into the influent of 2nd Vr to improve the total performance. The surface of 1st V and 2nd Vr was divided into two zones to maintain dry condition. Supersol and ALC were used as surface covering material. Bypass structure was also used in this system.

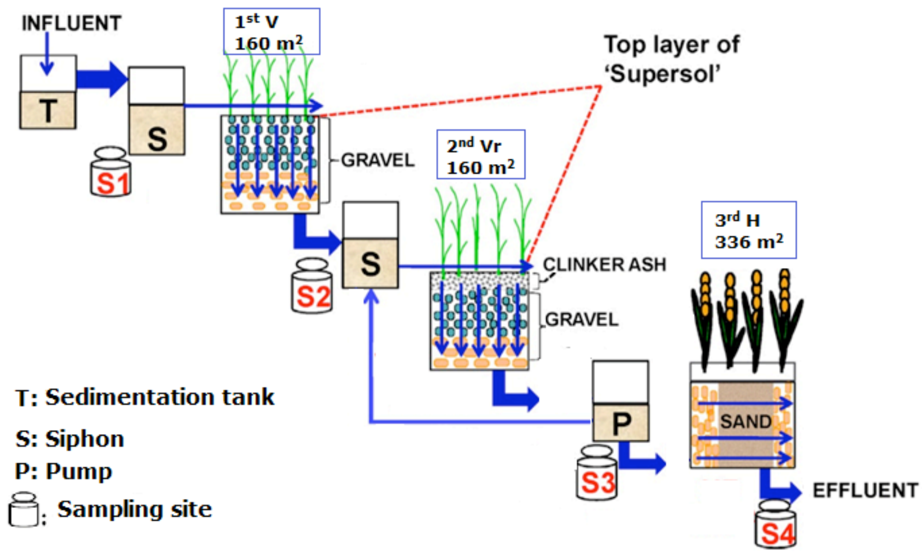

Fig. 2-5 Schematic diagram of Dairy-S hybrid system.

Total area of Diary-S was 656 m^2, with depth around 0.70 m. In the 1st V, river gravel was used as bed material, while it used a combination of river gravel and clinker ash as main bed material in 2nd Vr. Since the wastewater contains large amount of ammonia and organic nitrogen, it was most likely that the treated water acidifies due to nitric acid that was produced from nitrification, hence clinker ash was installed in the 2nd Vr to provide alkalinity. While in 3rd H, sand was used as bed material. More information of bed type and area, depths, main bed and surface covering material, and vegetation was shown in table 2-5. Common reed (*Phragmites australis*) was the main vegetation planted 1st V and 2nd Vr, and besides reed, other plant such as rice for experiment, cattails and other weed existed in 3rd H.

Table 2-5 Bed type and area, surface cover and main bed material, and vegetation of Dairy-S.

System	Bed type	Bed area (m^2)	Depth (m)	Main bed material	Surface covering material	Main vegetation
Dairy-S	1st V	160	0.75	River gravel	Supersol	Phragmites
	2nd Vr	160	0.71	Clinker ash; river gravel	Supersol	Phragmites
	3rd H	336	0.72	Sand	ALC	Phragmites; others

Note: ALC: autoclaved lightweight aerated concrete.

2.3.2 Hydraulic load

Piggery O's average hydraulic loading rate was 0.8 cm•d^{-1}. In summer period, the recirculation frequency from the 3rd V into the 1st Vr was once every 3 hours and lasted for 30 minutes each, while it was same frequency to dose wastewater from the 3rd V into the 2nd Vr but lasted for 90 minutes for each pumping. In winter period, the recirculation frequency from the 3rd V into the 1st Vr was once everyday and lasted for 20 minutes each, while it was also once a day from the 3rd V into the 2nd Vr but lasted for 30 minutes. The recirculation rate of effluent pumped from the 3rd V into the 1st Vr was 140 % and it was 80% in the 2nd Vr. Dairy G's average hydraulic loading rate was 1.1 cm•d^{-1}, while the recirculation scheduled only 15 minutes once a day, with a recirculation rate of 10%. Dairy S's average hydraulic loading rate was 0.7 cm•d^{-1}, and nearly 50% of effluent from 2nd Vr was dosed into the influent of same bed, with recirculation of 12 times per day, lasting 1 hour each time.

2.4 Sampling and analysis

Water samples were collected at the inlet of each bed and the final outlet, either monthly or bimonthly. At the sampling time, bottles were thoroughly rinsed with water to be sampled, and environmental parameters such as pH, electrical conductivity (EC), DO, oxidation-reduction potential (ORP), and water temperature (T) was recorded in situ during field measurement. After sampling, water quality indicators such as biochemical oxygen demand (BOD_5), SS, and total coliform (T. Coli.) were analyzed immediately. Water samples for total carbon (TC), COD, Total N, ammonium-N (NH_4-N), Nitrate-N (NO_3-N), Nitrite-N (NO_2-N), Organic-N (Org-N), Total phosphorus (T-P), phosphate (PO_4-P), and Organic-P (Org-P) were stored in a refrigerator for laboratory analysis.

In the lab, SS was measured by suction filtration method (filtration at 45μm and drying at 105°C) (APHA 1992). T-N and T-C were measured with an elemental analyzer (Elementar vario MAX; Elementar Analysensysteme GmbH, Hanau, Germany). NH_4-N was measured using a segmented-flow analysis system (QuAAtro; SEAL Analytical GmbH, Norderstedt, Germany). Org-N was calculated by subtracting the inorganic nitrogen (NH_4-N and NO_3-N) from T-N. T-P was measured with a colorimeter using the molybdenum blue ascorbic acid reduction method after decomposition by peroxodisulfate, PO_4-P was measured according to molybdenum blue visual colorimetric method (JIS K0102 46.3.1, Japan). COD was measured by spectrophotometer (DR2800; Hach, Loveland, CO) using a digital reactor (Hach DRB200) and disposable COD digestion vials (Hach).

Analysis methods referred to Standard Methods for the Examination of Water and Wastewater (APHA, 1992), and Testing Methods for Industrial Wastewater (JIS, 1998).

2.5 Water flow

Water flow rate was calculated by measuring the changes in the siphon tank's water table positions. Water table was recorded every 10 mins using pressure-type water-level gauge equipped with a data logger (DL/N70; Sensor Technik. Sirnach (STS) AG, Sirnach, Switzerland or S&DL Mini Oyo Corp., Tokyo, Japan). Flow rate was adjusted to take into account of precipitation and evapotranspiration in each bed. For Piggery-O and Dairy-G, all data from each data logger were used to calculate the flow. But for Dairy-S, due to low flow and entrapment of garbage, development of biofilm and interference of insects, this system used only the water table change data from the first siphon tank and calculated rest of the flow by incorporating evapotranspiration and precipitation multiplied by the bed area. Potential evapotranspiration was calculated using the Penman method and precipitation data were retrieved using a tipping-bucked installed on site or from the AMeDMS of the Japan Meteorological Agency that near to the hybrid system.

2.6 Calculation

Purification efficiency (PE), removal efficiency (RE), and removal rate were used to evaluate treatment efficiency of these hybrid systems.

$$\textit{Purification efficiency (\%)} = (C_{in} - C_{out}) \times 100 / C_{in}$$

$$\textit{Removal efficiency (\%)} = (L_{in} - L_{out}) \times 100 / L_{in}$$

$$\textit{Removal rate } (g \bullet m^{-2} \bullet d^{-1}) = L_{in} - L_{out}$$

$$L\ (g \bullet m^{-2} \bullet d^{-1}) = (\textit{Concentration} \times \textit{Flow rate}) / \textit{Bed area}$$

Where, C_{in} and C_{out} are pollutant concentrations in influent and effluent, respectively. L is pollutant load in wastewater, while L_{in} and L_{out} are the pollutant loads in influent and effluent, respectively, with a unit of $g \cdot m^{-2} \cdot d^{-1}$.

Chapter 3 Performance of treatment efficient of hybrid systems

3.1 Piggery-O

3.1.1 System running conditions

Environmental parameters such as pH, DO, ORP, EC and water temperature, as well as flow rate at the inlet of each bed and final outlet of Piggery-O system was shown in table 3-1. Compared with air temperature, water temperature in these systems varied from 10.9 ± 8.0°C to 16.4 ± 7.9°C. The ORP at each inlet and final outlet was normally positive, with an average value around 136 ± 143 mV to 335 ± 88 mV, which created conditions for N reduction, and there was an increasing tendency from the 1st Vr to 5th V. Piggery-O's pH values ranged from 6.9 ± 0.8 to 8.3 ± 0.5, which was suitable for nitrification. Totally, a slight decreased pH tendency existed in this system, which could relate to H^+ or HCO_3^- produced in N nitrification or denitrification, which decreased pH value. DO varied between 2.3 ± 2.6 to 4.6 ± 2.7 mg•L^{-1} in piggery O, and EC decreased from 10.8 ± 2.8 to 3.8 ± 0.6 mS•cm^{-1}, cause that the inflow wastewater was concentrated. Piggery-O inflow rate was around 11.6 ± 7.1 m^3•d^{-1}.

Table 3-1 Environmental parameters and flow rate at the inlet of each bed and final outlet of Piggery-O.

		1st Vr	2nd Vr	3rd V	4th H	5th V	Out
pH		8.3±0.5	8.0±0.5	7.7±0.5	7.7±0.5	7.5±0.6	6.9±0.8
T	°C	16.4±7.9	12.9±7.5	11.8±7.3	11.3±7.7	11.1±7.6	10.9±8.0
DO	mg•L^{-1}	2.3±2.6	3.3±2.8	3.7±2.0	4.3±2.4	3.7±2.7	4.6±2.7
ORP	mV	136±143	225±102	248±95	265±101	294±88	335±88
EC	mS•cm^{-1}	10.8±2.8	6.2±1.5	5.1±0.7	4.8±0.7	4.3±0.6	3.8±0.6
Flow rate	m^3•d^{-1}	11.6±7.1	26.9±17.5	42.1±25.9	11.8±6.7	12.0±6.9	12.1±7.0

Note: Average values represent mean ± standard deviation.

3.1.2 Pollutant purification efficiency

Piggery-O was monitored and evaluated during six years of operation. Table 3-2 shows pollutant concentration at the inlet of each bed and final outlet, along with overall purification efficiency. Totally, Piggery-O performed high T-N purification efficiency although it received high content wastewater, and the concentration decreased from 1,433 ± 342 mg•L^{-1} to 402 ± 104 mg•L^{-1}, with purification efficiency around 71 ± 9%. This value is similar with the one reported by Vymazal and Kröpfelová (2015). T-N concentration in all systems decreased gradually after wastewater passed through each bed, except that T-N concentration almost remained same after passed through the 5th V. This was reasonable because that although NH_4-N was transformed into NO_3-N, N was not released into atmosphere in form of N gaseous since denitrificaiton didn't occur efficiently in 5th V. As a result, N still remained in the wastewater. The treatment tendency of NH_4-N was similar with T-N treatment at each bed. NH_4-N concentration also decreased gradually at each bed and the purification efficiency was 82 ± 14%. For T-P, the average inflow concentration was 145 ± 148 mg•L^{-1} while the final outflow concentration was 14 ± 5 mg•L^{-1}, with an average purification of 91 ± 6%. Both Org-P and PO_4-P existed in the wastewater had a decreased tendency after wastewater passed through the whole system. COD and BOD_5 performed purification efficiency of 94 ± 6% and 98 ± 4%, respectively. T-C's purification efficiency was 91 ± 8%. Purification efficiency of T. Coli and SS was nearly 100%, and most of them were reduced sharply after passed through the 1st Vr, this fact has been reported by others (Vymazal and Kröpfelová, 2015).

Table 3-2 Pollutant concentration in the inflow of each bed and final outflow and the purification efficiency (PE) of Piggery-O.

		1st Vr	2nd Vr	3rd V	4th H	5th V	Out	PE (%)
T-N	mg•L^{-1}	1,433±342	666±206	511±146	473±131	397±103	402±104	72±9
NH_4-N	mg•L^{-1}	1,099±455	471±237	314±140	263±131	210±117	124±100	89±14
T-P	mg•L^{-1}	148±59	37±24	29±12	25±10	20±8	14±5	91±6
COD	mg•L^{-1}	6,340±3,396	1,687±1,166	999±587	826±444	598±262	411±200	94±6
BOD_5	mg•L^{-1}	1,914±1,693	413±427	163±125	127±91	80±52	45±34	98±4
SS	mg•L^{-1}	1,640±1,267	213±247	124±132	88±80	39±24	34±30	98±13
T.Coli.	CFU•100mL^{-1} (×10^3)	2,897±4,258	146±268	48±47	41±107	19±23	11±27	100±166
T-C	mg•L^{-1}	3,073±1,352	1,088±580	736±331	604±293	472±213	293±172	90±8
PO_4-P	mg•L^{-1}	52±27	16±10	17±6	16±5	15±6	11±5	79±11
Org-P	mg•L^{-1}	96±52	20±16	15±24	9±7	6±5	2±2	97±4
Org-N	mg•L^{-1}	313±285	110±124	119±119	117±109	75±73	82±84	74±18
NO_3-N	mg•L^{-1}	33±51	78±61	86±63	96±68	112±65	193±62	-

Note: Average values represent mean ± standard deviation.

The composition of N and P in the inflow of each bed and final outflow is shown in Fig. 3-1. NH_4-N took account of a large amount of T-N in the original inflow wastewater, and this percentage decreased from 79 % to 31% after wastewater passed through each bed, meanwhile, the concentration of NO_3-N increased after passing through each bed gradually, and the composition varied from 2% to 48%. This means N nitrification in this system performed efficiently. In addition, the amount of NO_3-N increased was not proportional with the amount of NH_4-N decreased, which indicated that denitrification also existed in the hybrid system, by which NO_3-N was transferred into N gaseous. For P, both Org-P and PO_4-P occupied large amount of T-P in the original inflow wastewater, and this amount decreased sharply after passed through the 1^{st} Vr. And then, Org-P decreased gradually after passed through the other beds, while for PO_4-P, it didn't decrease obviously.

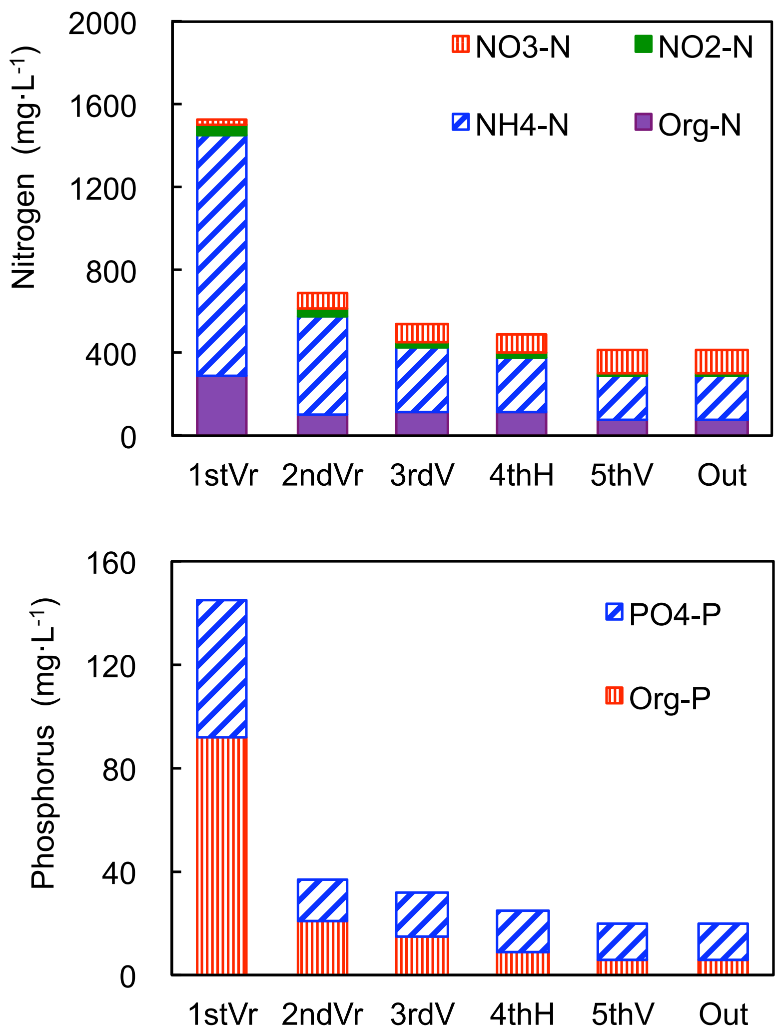

Fig. 3-1 The composition of N and P in the inflow of each bed and final outflow of Piggery-O.

3.1.3 Pollutant load, removal rate and removal efficiency

Pollutant's original inflow load, final outflow load, removal rate and removal efficiency of whole Piggery-O system is shown in table 3-3. In Piggery-O, the inflow T-N load was 11.2 ± 7.5 g•m^{-2}•d^{-1}, while the removal rate was 7.9±7.3 g•m^{-2}•d^{-1}, with T-N removal efficiency 71 ± 20%. The T-N removal rate was higher than the average removal rate of 4.2 ± 5.1 g•m^{-2}•d^{-1} reported in Vymazal's review (2013). The NH_4-N removal rate was 8.1 ± 5.4 g•m^{-2}•d^{-1}. For T-P, the influent load was 1.2 ± 0.9 g•m^{-2}•d^{-1}, while the removal rate was 1.0 ± 0.9 g•m^{-2}•d^{-1}, with removal efficiency of 90 ± 10%. The whole system received high load of T-C, COD and BOD_5, and it performed good removal efficiency of 88 ± 13%, 93 ± 10%, and 97 ± 7%, respectively. The removal efficiency of SS and T.Coli reached nearly 100%. These removal efficiencies indicated Piggery-O system performed well for pollutants removal.

Table 3-3 Pollutant load in the original inflow (load in) and final outflow (load out), total removal rate, and removal efficiency of Piggery-O.

		T-N	NH_4-N	T-P	COD	BOD_5	SS
Load in	g•m^{-2}•d^{-1}	11.2±7.5	8.1±5.4	1.2±0.9	53.5±52.1	16.5±18.9	12.6±12.3
Load out	g•m^{-2}•d^{-1}	3.2±1.9	1.0±1.0	0.1±0.1	3.8±3.6	0.4±0.5	0.3±0.4
Removal rate	g•m^{-2}•d^{-1}	7.9±7.3	7.1±5.3	1.0±0.9	49.7±50.4	16.1±18.7	12.3±12.2
Removal efficiency	%	71±20	87±22	90±10	93±10	97±7	98±5
		T-C	PO_4-P	Org-P	Org-N	NO_3-N	
Load in	g•m^{-2}•d^{-1}	23.8±20.1	0.4±0.3	0.8±0.7	2.9±3.6	0.3±0.5	
Load out	g•m^{-2}•d^{-1}	2.8±3.1	0.1±0.1	0.1±0.1	0.8±1.0	1.4±0.7	
Removal rate	g•m^{-2}•d^{-1}	21.0±18.4	0.3±0.3	0.7±0.7	2.1±3	-1.1±0.8	
Removal efficiency	%	88±13	76±22	97±6	74±22	-	
		T.Coli.					
Load in	CFU•m^{-2}•d^{-1}	211±331					
Load out	CFU•m^{-2}•d^{-1}	1±2					
Removal rate	CFU•m^{-2}•d^{-1}	210±331					
Removal efficiency	%	100±165					

Note: Average values represent mean ± standard deviation.

Fig. 3-2 shows average inflow load and outflow load, removal rate as well as removal efficiency at each bed of Piggery-O. According to this, we can see the received load and removal efficiency of each bed. For T-N, the removal rate decreased from 14.5 ± 13.9 g•m^{-2}•d^{-1} in the 1st Vr to -0.6 ± 5.6 g•m^{-2}•d^{-1} in the 5th V. Similarly, the 1st Vr performed highest removal efficiency around 51 ± 19%, while the 5th V performed negative removal efficiency. For T-P, the 1st Vr had highest removal efficiency of 71 ± 17% while it received a load of 3.0 ± 2.3 g•m^{-2}•d^{-1}. All five beds performed positively for T-P removal, and the 3rd V received a high load and performed low removal efficiency. For T-C removal, all beds received loads between 39.5 ± 38.3 g•m^{-2}•d^{-1} and 163.7 ± 150.1 g•m^{-2}•d^{-1}, this was higher than the loads reported by Vymazal (2013), and nearly 59 ± 23% of received T-C was removed by the 1st Vr, although this removal efficiency decreased in the following four beds, the system still performed a high value. For COD and BOD_5, the 1st Vr had highest removal efficiency, and the 3rd V had the lowest value. Totally, the 3rd V performed lower removal efficiency compared with other beds, and the 1st Vr and 2nd Vr removed a large amount of pollutants received.

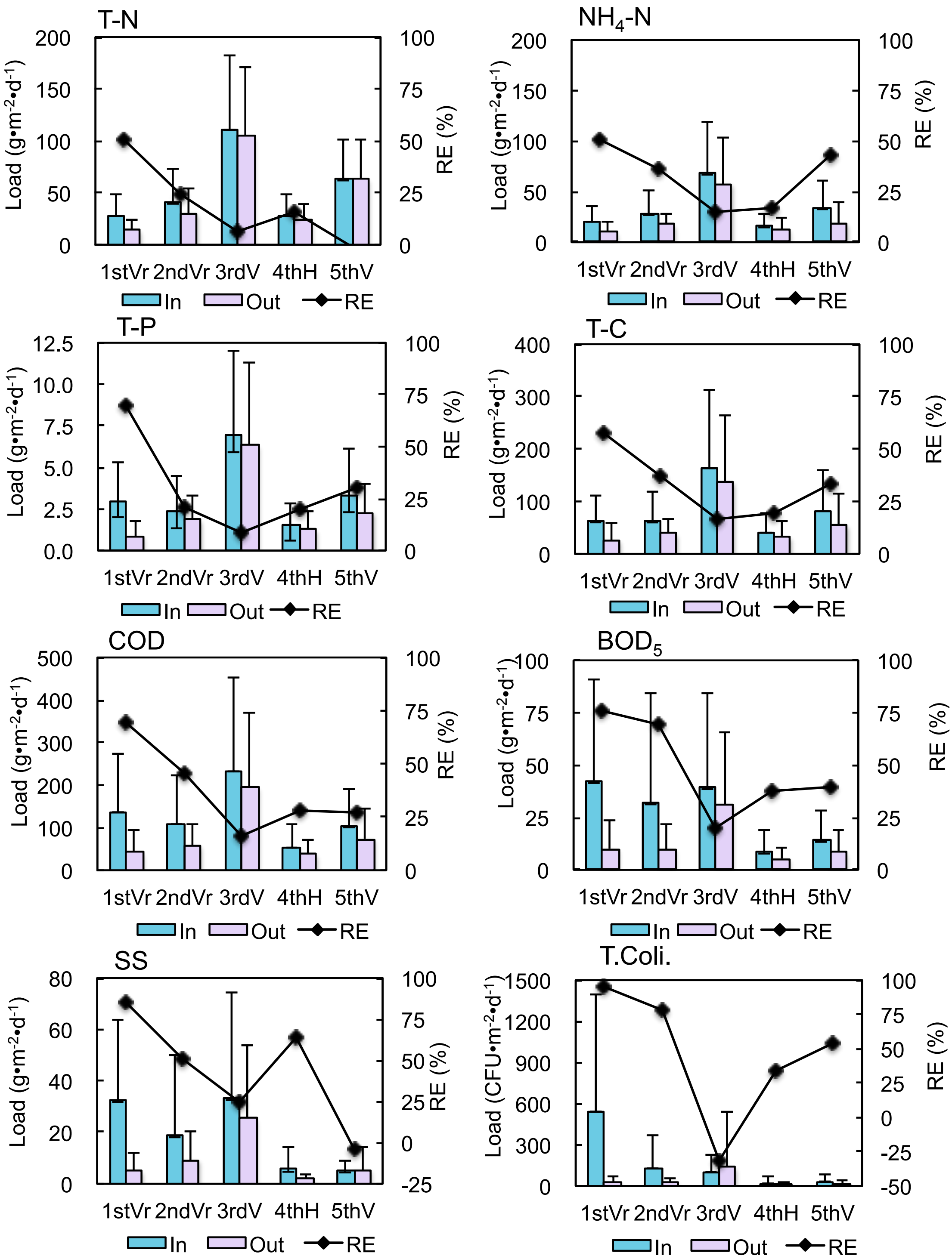

Fig. 3-2 Pollutant's inflow (in) load and outflow (out) load, as well as removal efficiency at each bed of Piggery-O.

3.1.4 Yearly removal performance

Fig. 3-3 shows annual inflow load and final outflow load of T-N, NH_4-N, T-P, T-C, COD, BOD_5, SS, and T.Coli. in form of box-and-whisker diagrams, along with annual removal efficiency of Piggery-O.

Totally, the yearly received T-N was around 7.0 ~ 21.5 $g•m^{-2}•d^{-1}$. In the first year, T-N' removal efficiency was not high, after one year's operation, it increased to 66%, and maintained at 62 ~ 88% in the following years. The NH_4-N performed stable and high removal efficiency ranged 80 ~ 90 % after one year's operation. For T-P, the system presented high removal efficiency of 80% from the beginning of construction. This could be attributed to the use of pumice gravel as the bed material. Pumice gravel can potentially enhance treatment efficiency of P due to its high P adsorption capacity. The removal of organic matter such as T-C, COD and BOD_5 in this system also performed high value in the first year, and the removal efficiency of them reached 90% in the second year, and maintained stable since then. These could be explained by an increase of microorganisms involved in N transformation and organic removal after years of construction and the gradual improvement of conditions for pollutants removal. Overall, the hybrid CW system had increased pollutant removal efficiency each year.

The whole system also performed high annual removal efficiency in SS and T. Coli. from the beginning of operation. Totally, for all pollutants, the system performed increasing removal efficiency, and it presented stable and high performance in recent years.

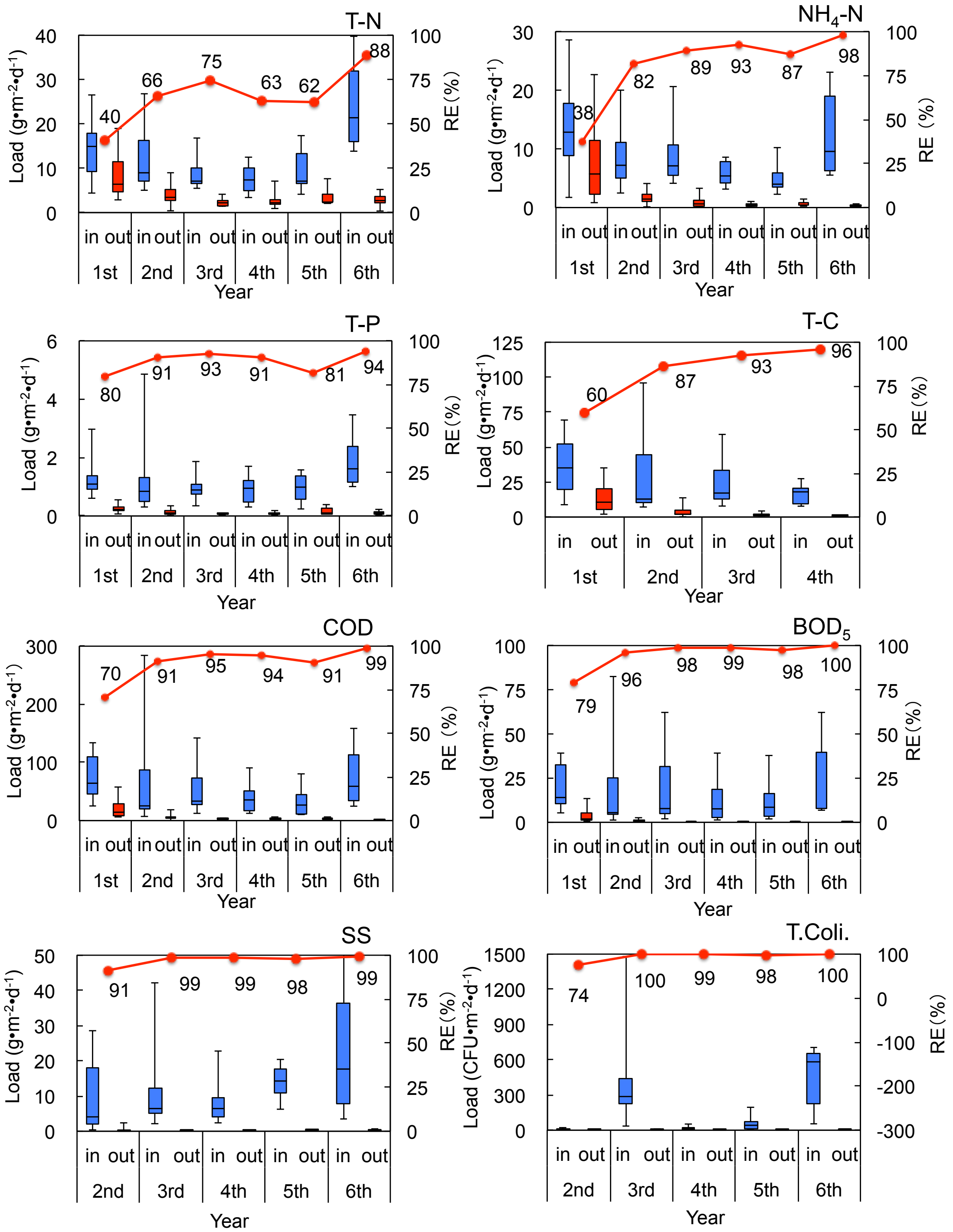

Fig. 3-3 Yearly pollutant inflow load (in) and outflow load (out), and the yearly removal efficiency (RE) of Piggery-O.

3.1.5 Pollutant removal efficiency in cold and warm period

Fig. 3-4 shows Piggery-O's removal efficiency during the cold period (from Nov. to Apr. of the following year) and warm period (from May to Oct.), with respect to T-N, NH_4-N, T-P, T-C, COD, BOD_5, SS, and T. Coli. at each bed.

In the 1st Vr, it performed high removal efficiency for all the pollutants during both cold and warm period. In the 2nd Vr bed, average removal efficiency for all pollutants was higher in warm period than in cold period. But in the 3rd bed, for T-N, NH_4-N, and TP, it had higher removal efficiency in cold period than warm period. That's because in warm period, 3rd V received a large amount of pollutant load compared with in cold period. Hence the calculated removal efficiency was lower in cold period. But for COD and BOD_5, the removal efficiency was similar in both periods even it received high influent load in cold period, this was associated with that high influent load would load to high removal (Kato *et al.*, 2013a,b). In the 4th H bed, removal efficiency for T-N, NH_4-N, T-C, COD, BOD_5, and SS was higher during the warm period. In the 5th V bed, removal efficiency for NH_4-N, T-P, T-C, COD, and BOD_5 was higher during the warm period. Totally, N, T-C, COD, and BOD_5 removal varies in both warm period and cold period. This could be explaied that processes such as ammonification, nitrification, and denitrification involve temperature dependant microbial activities, so the removal efficicency can vary by season (Kadlec, 2000).

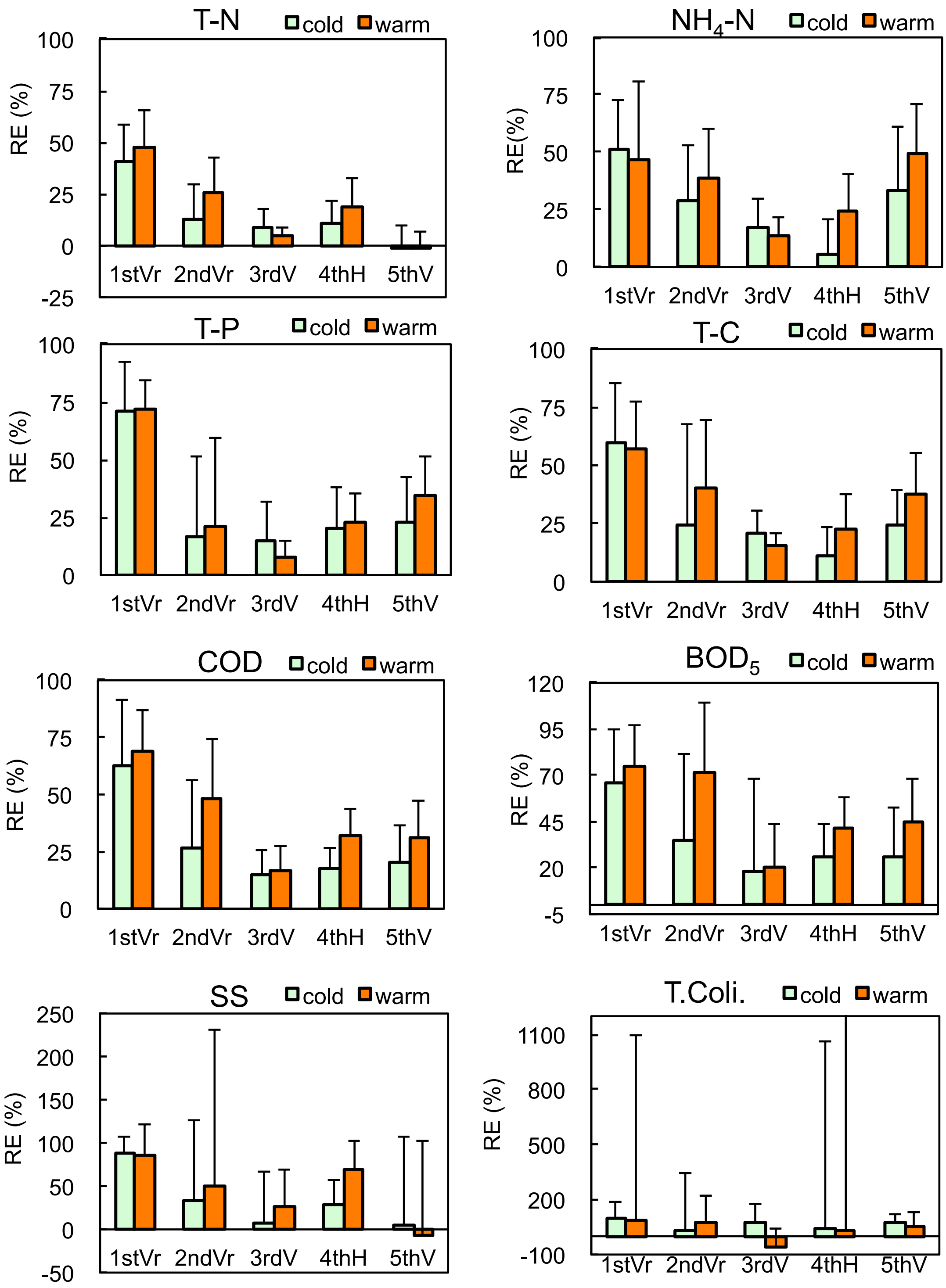

Fig. 3-4 Pollutant's removal efficiency (RE) in cold and warm period at each bed of Piggery-O.

3.2 Dairy-G

3.2.1 System running conditions

Environmental parameters such as pH, DO, ORP, EC and water temperature as well as flow rate at the inflow of each bed and final outflow of Dairy-G system was shown in table 3-4. Water temperature in these systems varied from 8.9 ± 5.4˚C to 13.1 ± 4.3˚C. The ORP at each inlet and final outlet was normally positive, with an average value around 181 ± 60 ~ 273 ± 65 mV, and the lowest value presented in the influent of 4th H. The ORP condition was suitable for organic matter and N decomposition. In CWs, process of organic pollutants break down depends on redox (oxidation-reduction) conditions. High redox potential is associated with oxidized environment and promotes aerobic processes such as nitrification. pH values at each bed ranged from 6.0 ± 0.3 to 6.8 ± 0.3. The pH dropped after wastewater passed through the 1st Vr, that might be a large amount of NH_4-N was nitrated, which reduced the pH. The DO varied between 2.1 ± 1.4 ~ 4.7 ± 2.3 mg•L^{-1}, and highest value existed in the 1st Vr. EC was as around 0.8 ± 0.2 mS•cm^{-1}. The original inflow flow rate of this system around 33.2 ± 6.9 m^3•d^{-1}.

Table 3-4 Environmental parameters and flow rate in the inflow of each bed and final outflow of Dairy-G.

		1stVr	2ndV	3rdV	4thH	5thV	Out
pH		6.4±0.9	6.0±0.3	6.4±0.2	6.6±0.2	6.8±0.2	6.8±0.3
T	°C	13.1±4.3	11.2±5.1	10.8±5.4	10.9±6.3	8.9±5.7	8.9±5.4
DO	mg•L^{-1}	4.7±2.3	2.6±1.4	3.0±1.7	2.1±1.4	2.7±1.5	2.2±1.4
ORP	mV	236±32	214±49	193±58	181±60	205±66	273±65
EC	mS•cm^{-1}	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2	0.9±0.2	0.8±0.2
Flow rate	m^3•d^{-1}	33.2±6.9	37.6±8.6	37.1±6.9	39.5±8.4	40.3±8.7	40.6±9.0

Note: Average values represent mean ± standard deviation.

3.2.2 Pollutant purification efficiency

Dairy-G was monitored and evaluated during the five years of operation. Table 3-5 shows pollutant concentration at the inflow of each bed and final outflow, along with overall purification efficiency in Dairy-G. Totally, Dairy-G performed high purification efficiency although it received high content wastewater, especially a high level of COD of 10,961 ± 3,146 mg•L^{-1}, which was much higher than inflow concentrations listed in Vymazal's review (2013). T-N concentration decreased gradually after wastewater passed through each bed, with overall purification efficiency around 85 ± 7%. The final effluent T-N concentration was 45 ± 24 mg•L^{-1}, which was below the 60 mg•L^{-1} thresholds set by Japanese water quality regulators. The treatment tendency of NH_4-N was different with T-N treatment tendency. Dairy-G's NH_4-N concentration initially increased after passed through the 1st Vr, and later decreased gradually in the subsequent four beds. Dairy-G's NH_4-N removal pattern may be attributed to the high content of Org-N in its influent. When incoming wastewater has high Org-N, ammonification initiates the first step of N transformation (nitrification) in the subsurface flow wetland systems (Saeed and Sun, 2012).

For T-P, the average inflow concentration was 60 ± 18 mg•L^{-1} while the final outflow concentration was 15 ± 6 mg•L^{-1}. Org-P and PO_4-P decreased after passing through each bed, and had a purification efficiency of 64 ± 34% and 77 ± 10%, respectively. Dairy-G presented purification efficiency of 99 ± 1% for SS, while for T.Coli., it was 98 ± 145%.

Dairy-G presented high purification efficiency for COD and BOD_5 over 96 ± 2% and 98 ± 1%. This indicated that this kind of hybrid system could perform well to treat high organic content wastewater.

The composition of N and P in the inflow of each bed and final outflow is shown in Fig. 3-5. Org-N took account of higher N composition in the original inflow wastewater, and this percentage decreased after wastewater passed through each bed, meanwhile, the content of NH_4-N increased after passed though the 1st V bed. NO_3-N concentration didn't vary much in the first four beds; this might indicated both nitrification and denitrifcation worked effectively. Similar with Piggery-O system, NO_3-N concentration increased after the wastewater passed through the 5th V. In Dairy-G, PO_4-P was dominant in the original influent, and it decreased greatly after passed through the 1st Vr.

Table 3-5 Pollutant concentration in the inflow of each bed and final outflow, and the purification efficiency (PE) of Dairy-G.

		1^{st}Vr	2^{nd}V	3^{rd}V	4^{th}H	5^{th}V	Out	PE (%)
T-N	mg•L^{-1}	300±92	106±37	89±38	65±25	58±28	45±24	85±7
NH_4-N	mg•L^{-1}	9±4	31±13	32±17	28±15	33±16	18±15	-97±255
T-P	mg•L^{-1}	60±18	26±7	23±7	20±6	17±7	15±6	75±9
COD	mg•L^{-1}	10,961±3,146	2,794±1,035	1,847±704	1,076±443	596±274	418±184	96±2
BOD_5	mg•L^{-1}	7,093±3,076	1,487±634	972±485	450±273	206±167	132±98	98±1
SS	mg•L^{-1}	2,816±897	459±220	254±147	129±76	49±38	31±21	99±1
T.Coli.	CFU•100mL^{-1} (×10^3)	16,950±47,171	2,924±3,832	2,146±2,776	2,383±4,680	586±681	359±358	98±145
T-C	mg•L^{-1}	3,126±550	730±272	533±184	367±106	254±89	188±73	94±3
PO_4-P	mg•L^{-1}	40±10	17±5	14±5	12±4	11±4	9±3	77±10
Org-P	mg•L^{-1}	11±6	6±2	6±2	5±2	5±3	4±3	64±34
Org-N	mg•L^{-1}	291±92	74±35	57±36	37±19	25±15	23±13	92±4
NO_3-N	mg•L^{-1}	0.3±0.3	0.4±0.8	0.2±0.2	0.1±0.2	0.1±0.1	3.8±4.3	-

Note: Average values represent mean ± standard deviation.

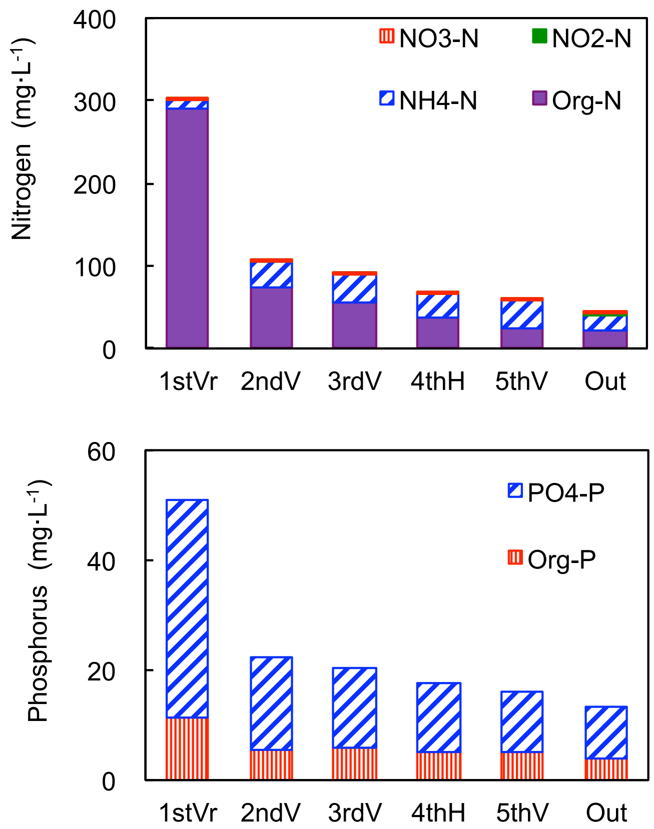

Fig. 3-5 The composition of N and P in the inflow of each bed and final outflow of Dairy-G.

3.2.3 Pollutant load, removal rate and removal efficiency

Original pollutant's inflow load, final outflow load, removal rate and removal efficiency of whole Dairy-G system is shown in table 3-6. In Dairy-G, the T-N inflow load was 3.4 ± 1.7 $g•m^{-2}•d^{-1}$, while the removal rate was 2.8 ± 1.4 $g•m^{-2}•d^{-1}$, with a removal efficiency of 81 ± 9%. For T-P, the influent loads were 0.7 ± 0.3 $g•m^{-2}•d^{-1}$, while the removal rate was 0.5 ± 0.2 $g•m^{-2}•d^{-1}$, with a removal efficiency of 70 ± 11%. The NH_4-N removal rate was negative in this system because that a large amount of NH_4-N was produced by decomposition of Org-N. Although the whole system received high inflow load of COD and BOD_5, it performed good removal rate of 118.4 ± 57.1 $g•m^{-2}•d^{-1}$ and 84.9 ± 52.2 $g•m^{-2}•d^{-1}$, with high removal efficiencies more than 95%. For T.Coli., this system had total removal efficiency around 80 ± 183%. The SS removal efficiency was nearly 100%.

High removal efficiency of BOD_5 and COD in this system might be concerned with efficiency degradation of organic matter in V bed, which provided oxidized conditions for aerobic degradation. Nitrification and denitrification was favoured because of the present of DO and rich content of T-C in the influent of the wastewater.

Fig. 3-6 shows pollutant average influent load and effluent load at each bed, as well as removal efficiency in Dairy-G. For T-N and T-P, the 1^{st} V performed high removal efficiency and received the high load. For T-C, COD, BOD_5, after high removal efficiency presented in 1^{st} V, other four beds had stable and similar removal efficiency. Totally, large amount of pollutants were removed in the 1^{st} V except NH_4-N.

Table 3-6 Pollutant load in the original inflow (load in) and final outflow (load out), total removal rate, and removal efficiency of Dairy-G.

		T-N	NH_4-N	T-P	COD	BOD_5	SS
Load in	g•m^{-2}•d^{-1}	3.4±1.7	0.1±0.0	0.7±0.3	124.0±58.5	86.8±52.9	33.7±15.8
Load out	g•m^{-2}•d^{-1}	0.6±0.4	0.3±0.2	0.2±0.1	5.6±2.8	1.9±1.5	0.5±0.3
Removal rate	g•m^{-2}•d^{-1}	2.8±1.4	-0.2±0.2	0.5±0.2	118.4±57.1	84.9±52.2	33.2±15.6
Removal efficiency	%	81±9	-151±280	70±11	95±2	98±2	99±1
		T-C	PO_4-P	Org-P	Org-N	NO_3-N	
Load in	g•m^{-2}•d^{-1}	29.6±6.1	0.4±0.2	0.1±0.1	3.3±1.7	0.0±0.0	
Load out	g•m^{-2}•d^{-1}	2.2±0.9	0.1±0.1	0.1±0.0	0.3±0.2	0.1±0.1	
Removal rate	g•m^{-2}•d^{-1}	27.4±6.1	0.3±0.1	0.1±0.1	3.0±1.5	-0.1±0.1	
Removal efficiency	%	93±3	71±12	58±38	90±6	-	
		T.Coli.					
Load in	CFU•m^{-2}•d^{-1}	2,640±7,426					
Load out	CFU•m^{-2}•d^{-1}	54±64					
Removal rate	CFU•m^{-2}•d^{-1}	2,106±6,764					
Removal efficiency	%	80±183					

Note: Average values represent mean ± standard deviation.

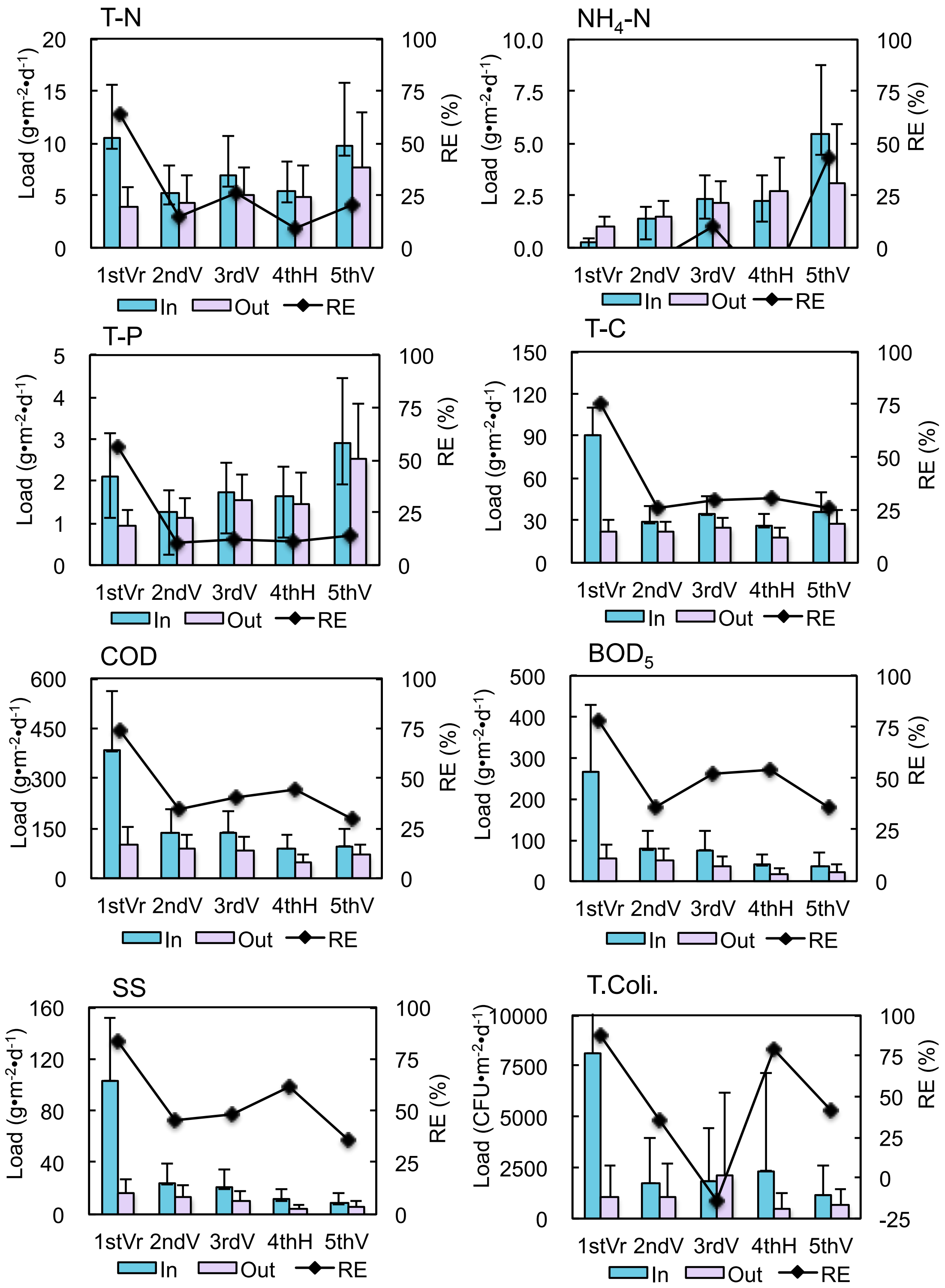

Fig. 3-6 Pollutant's inflow (in) load and outflow (out) load, as well as removal efficiency at each bed of Dairy-G.

3.2.4 Yearly removal performance

Fig. 3-7 shows the annual inflow load and final outflow load of T-N, NH_4-N, T-P, T-C, COD, BOD_5, SS, and T.Coli. in form of box-and-whisker diagrams, along with annual removal efficiency of Dairy-G.

From the first year to the forth year, the median value of received T-N load was in range of 2.17 - 2.99 $g \bullet m^{-2} \bullet d^{-1}$, and it was 0.24 - 1.47 $g \bullet m^{-2} \bullet d^{-1}$ in the effluent. Although the removal efficiency had a slightly decrease in latest two years, it was still in a high level. The system's removal efficiency of T-P also decreased from 80% in the first year to 69% in the forth year. This could be due to saturation of the river gravel's adsorption capacity over time. For T-C, COD, BOD_5, and SS, Dairy-G presented high removal efficiency from the beginning of operation. The removal of T.Coli. was not stable, this was because that the hybrid system might provide suitable condition for T. Coli. growth, hence the concentration of T. Coli. existed in this system varied greatly and as a result, the calculation value varied greatly.

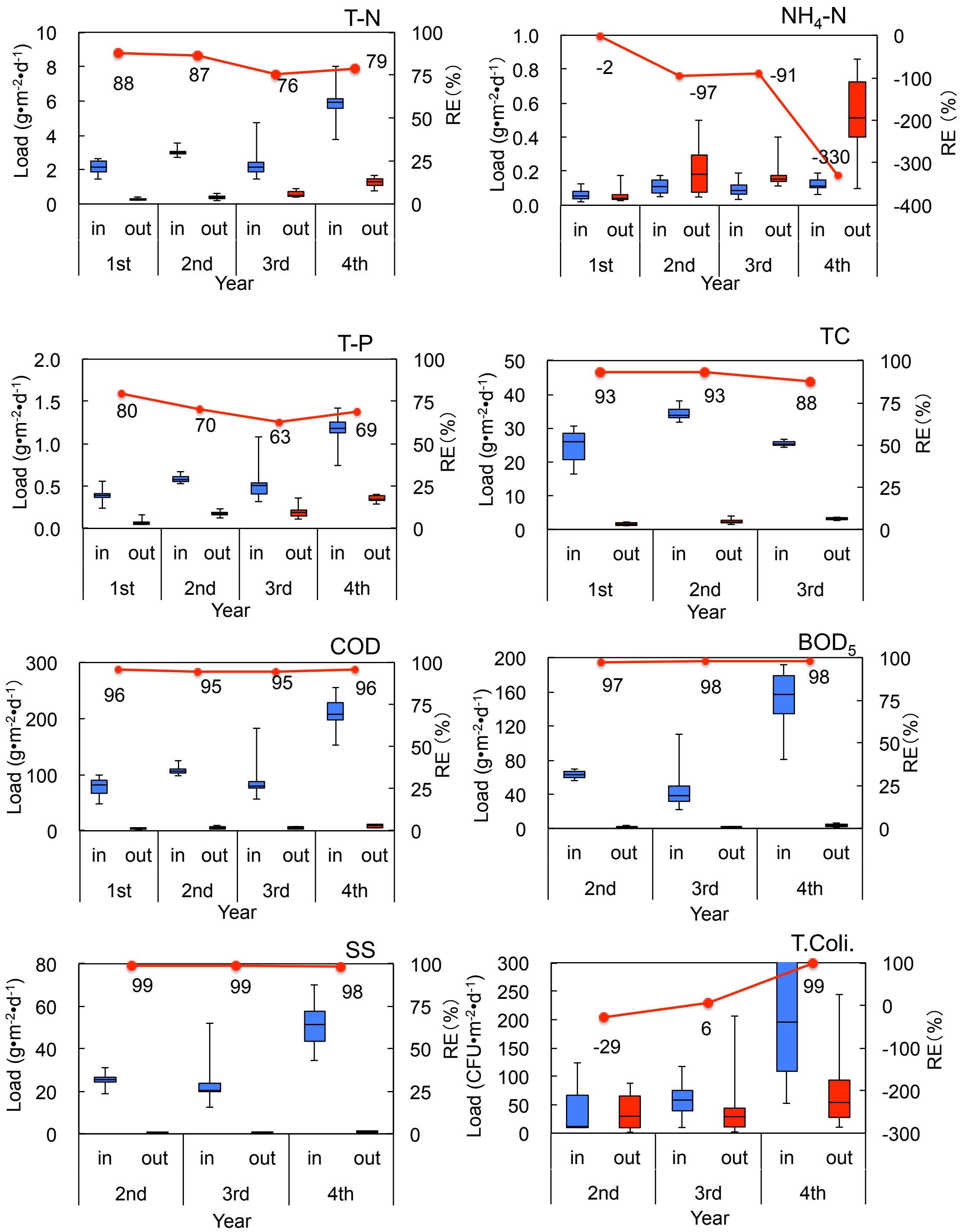

Fig. 3-7 Yearly pollutant's inflow load (in) and outflow load (out), and the yearly removal efficiency (RE) of Dairy-G.

3.2.5 Pollutant removal efficiency in cold and warm period

Fig. 3-8 shows the Dairy-G's removal efficiency with respect to T-N, NH_4-N, T-P, T-C, COD, BOD_5, SS, and T.Coli at each bed during the cold period and warm period.

In this system, the removal of pollutants didn't show much difference in both in warm period and in cold period, they all presented high removal efficiency in two periods except NH_4-N and T. Coli.. For NH_4-N, the removal efficiency was low, or even negative. And for T.Coli., the extremely higher value affected the averaged removal efficiency, as a result, the average removal efficiency of T.Coli. varied greatly.

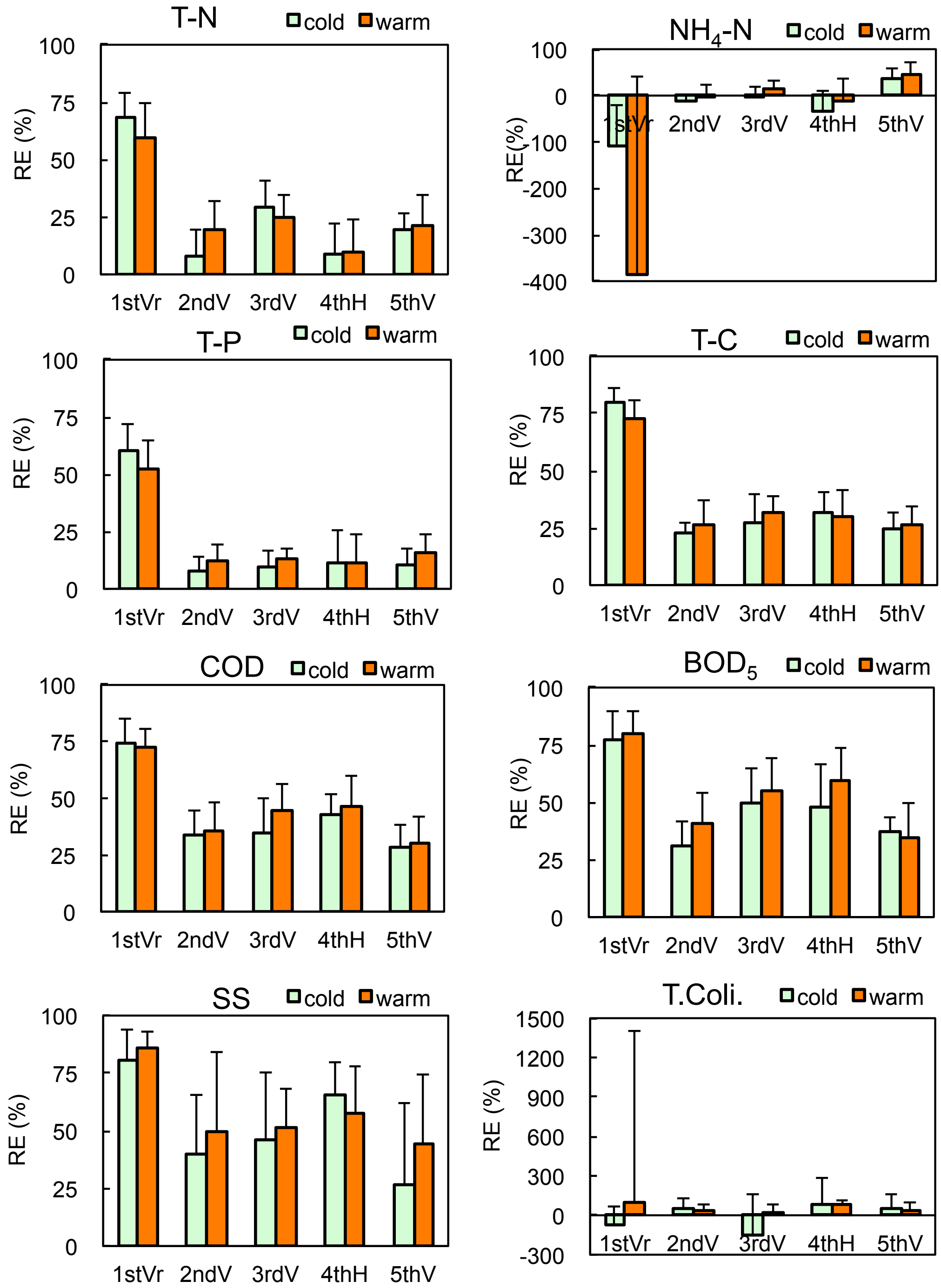

Fig. 3-8 Pollutant's removal efficiency (RE) in cold and warm period at each bed of Dairy-G.

3.3 Dairy-S

3.3.1 System running conditions

Environmental parameters such as pH, DO, ORP, EC and water temperature as well as flow rate in the inflow of each bed and final outflow of Dairy-S system was shown in table 3-7. Water temperature in these systems varied from 6.9 ± 5.3°C to 11.7 ± 4.6°C. The ORP was around 209 ± 68 mV to 255 ± 82 mV, which was suitable for nitrate reduction (Faulwetter *et al.*, 2009). pH maintained 6.6 ± 0.6 to 6.9 ± 0.5. The DO varied between 1.8 ± 1.2 and 2.0 ± 1.1 mg•L^{-1}, which was lower compared with the other two hybrid systems. The inflow flow rate of each bed was around 4.8 ± 0.9 ~ 6.5 ± 2.8 m^3 d^{-1}, compared with Dairy-G, Dairy-S received relatively lower water flow.

Table 3-7 Environmental parameters and flow rate in the inflow of each bed and final outflow of Dairy-S.

		1^{st} V	2^{nd} Vr	3^{rd} H	Out
pH		6.6±0.6	6.8±0.3	6.9±0.3	6.9±0.5
T	°C	11.7±4.6	10.6±5.5	9.4±5.8	6.9±5.3
DO	mg•L^{-1}	1.8±1.5	1.7±1.2	2.0±1.1	1.8±1.2
ORP	mV	209±68	222±63	215±68	255±82
EC	mS•cm^{-1}	1.3±0.3	1.3±0.3	1.1±0.3	0.9±0.2
Flow rate	m^3•d^{-1}	4.8±0.9	5.0±1.1	5.2±1.3	6.5±2.8

Note: Average values represent mean ± standard deviation.

3.3.2 Pollutant purification efficiency

Dairy-S was monitored and evaluated during nine years of operation. Table 3-8 shows pollutant concentration in the inflow of each bed and final outflow, along with overall purification efficiency of Dairy-S. The T-N concentration decreased gradually after wastewater passed through each bed. The final outflow T-N concentrations for Dairy-S was 22 ± 15 mg•L^{-1}, which also achieved the wastewater discharge standard set by Japanese water quality regulators. Total purification efficiency was around 86 ± 12% for T-N, which was higher than Piggery-O and Dairy-G. For T-P, the average inflow concentration was 26 ± 11 mg•L^{-1} while the final outflow concentration was 7 ± 3 mg•L^{-1}, with an average purification efficiency of 75 ± 28%. Dairy S's NH_4-N treatment tendency was similar with T-N treatment, NH_4-N concentration decreased gradually in the subsequent three beds, and NO_3-N increased after passed through the first two V bed, and then decreased after it passed through the 3rd H, where the 3rd H provided suitable condition for NO_3-N denitrification.

Dairy-S received 3,752 ± 2,071 mg•L^{-1} COD and 1,446 ± 590 mg•L^{-1} BOD_5, the purification efficiency was over 90%. T-C purification efficiency was 88 ± 8%. SS and T.Coli purification efficiency was near 100%. This indicated that this hybrid system performed well in high organic matter content wastewater purification.

The composition of N and P in the inflow of each bed and the final outflow is shown in Fig. 3-9. Org-N and NH_4-N both took account of large amount of T-N composition in the original inflow wastewater, and this percentage decreased after wastewater passed through each bed, the content of NO_3-N didn't increase as much as it increased in Piggery-O. PO_4-P occupied higher percentage of T-P compared with Org-P both in the influent and effluent, and the concentration of them decreased after passing through each bed.

Table 3-8 Pollutant's concentration in the inflow of each bed and final outflow, and the purification efficiency of Dairy-S.

		1st V	2nd Vr	3rd H	Out	PE (%)
T-N	mg•L^{-1}	159±60	83±35	47±29	22±15	86±12
NH_4-N	mg•L^{-1}	67±26	46±28	28±26	13±11	80±27
T-P	mg•L^{-1}	26±11	19±7	14±6	7±3	75±28
COD	mg•L^{-1}	3,752±2,071	1,151±824	539±466	208±190	94±5
BOD_5	mg•L^{-1}	1,446±590	689±359	274±164	88±78	94±4
SS	mg•L^{-1}	652±478	126±139	45±56	13±15	98±4
T.Coli.	CFU•100mL^{-1} (×10^3)	11,870±22,559	5,884±10,248	1,406±1,574	112±232	99±10
T-C	mg•L^{-1}	1,192±621	450±269	255±168	140±72	88±8
PO_4-P	mg•L^{-1}	21±10	16±6	12±5	5±3	75±63
Org-P	mg•L^{-1}	5±2	3±1	2±2	1±1	75±19
Org-N	mg•L^{-1}	91±50	33±20	16±10	8±6	91±8
NO_3-N	mg•L^{-1}	0.3±0.5	4.4±11.4	2.8±5.1	1.1±1.6	-

Note: Average values represent mean ± standard deviation.

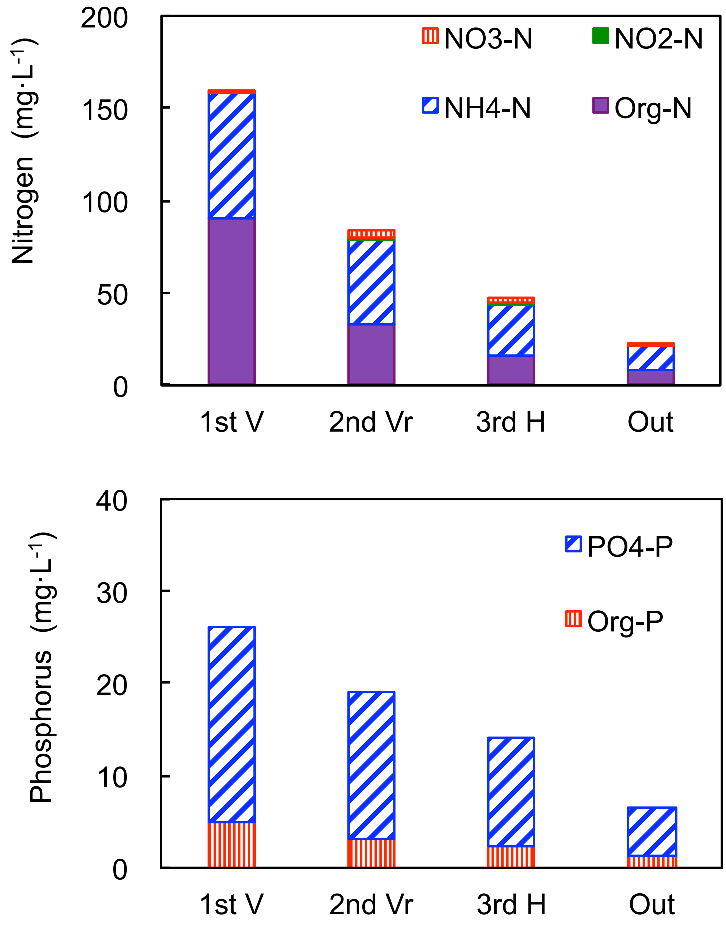

Fig. 3-9 The composition of N and P in the inflow and final outflow at each bed of Dairy-S.

3.3.3 Pollutant load, removal rate and removal efficiency

Pollutant's original inflow load, final outflow load, removal rate and removal efficiency of whole Dairy-S hybrid system is shown in table 3-9. In Dairy-S, the inflow T-N load was 1.2 ± 0.5 $g•m^{-2}•d^{-1}$, with removal efficiency of 82 ± 15%. For T-P, the inflow load was 0.2 ± 0.1 $g•m^{-2}•d^{-1}$, while the removal efficiency was 64 ± 29%, which was lower than that of Piggery-O, this might be involved of the bed material used in this system, compared with pumice gravel, the sand used in Diary-S had less P absorption ability. The NH_4-N removal efficiency was 74 ± 28%. The whole system received inflow load COD of 27.6±15.8 $g•m^{-2}•d^{-1}$ and it performed good removal efficiency of 93 ± 8%. For BOD_5, the whole system received load of 10.5 ± 4.2 $g•m^{-2}•d^{-1}$, with the removal efficiency of 92 ± 7%. Totally, SS and T.Coli. had removal efficiency reached nearly 100%.

Fig. 3-10 shows average inflow load and outflow load, as well as removal efficiency at each bed of Dairy-S. From this, we can see the received load and removal efficiency of each bed obviously. The 1st V, 2nd Vr, and 3rd H received decreasing influent load of each pollutant, and they presented stable removal efficiency for T-N, NH_4-N, and T-P although there was slightly increase or decrease. For T-C, COD and SS, the removal efficiency of each bed decreased slightly, while for BOD_5, the removal efficiency increased slightly. Totally, all beds performed positive and relatively stable removal efficiency for pollutant removal except T. Coli.. The T.Coli. presented obviously increased removal efficiency after passing through three beds.

Table 3-9 Pollutant load in the original inflow (load in) and final outflow (load out), removal rate, and removal efficiency of Dairy-S.

		T-N	NH_4-N	T-P	COD	BOD_5	SS
Load in	g•m^{-2}•d^{-1}	1.2±0.5	0.5±0.2	0.2±0.1	27.6±15.8	10.5±4.2	4.8±3.5
Load out	g•m^{-2}•d^{-1}	0.2±0.2	0.1±0.1	0.1±0.1	2.0±1.9	0.8±0.9	0.1±0.2
Removal rate	g•m^{-2}•d^{-1}	1.0±0.5	0.4±0.2	0.1±0.1	25.6±15.3	9.7±4.0	4.6±3.5
Removal efficiency	%	82±15	74±28	64±29	93±8	92±7	97±5
		T-C	PO_4-P	Org-P	Org-N	NO_3-N	
Load in	g•m^{-2}•d^{-1}	8.5±4.7	0.2±0.1	0.04±0.02	0.7±0.4	0.0±0.0	
Load out	g•m^{-2}•d^{-1}	1.2±0.7	0.1±0.0	0.01±0.01	0.1±0.1	0.01±0.02	
Removal rate	g•m^{-2}•d^{-1}	7.3±4.4	0.1±0.1	0.02±0.02	0.6±0.4	-0.01±0.02	
Removal efficiency	%	86±9	63±42	64±33	88±13	-	
		T.Coli.					
Load in	CFU•m^{-2}•d^{-1}	802±1,478					
Load out	CFU•m^{-2}•d^{-1}	15±45					
Removal rate	CFU•m^{-2}•d^{-1}	787±1,474					
Removal efficiency	%	100±165					

Note: Average values represent mean ± standard deviation.

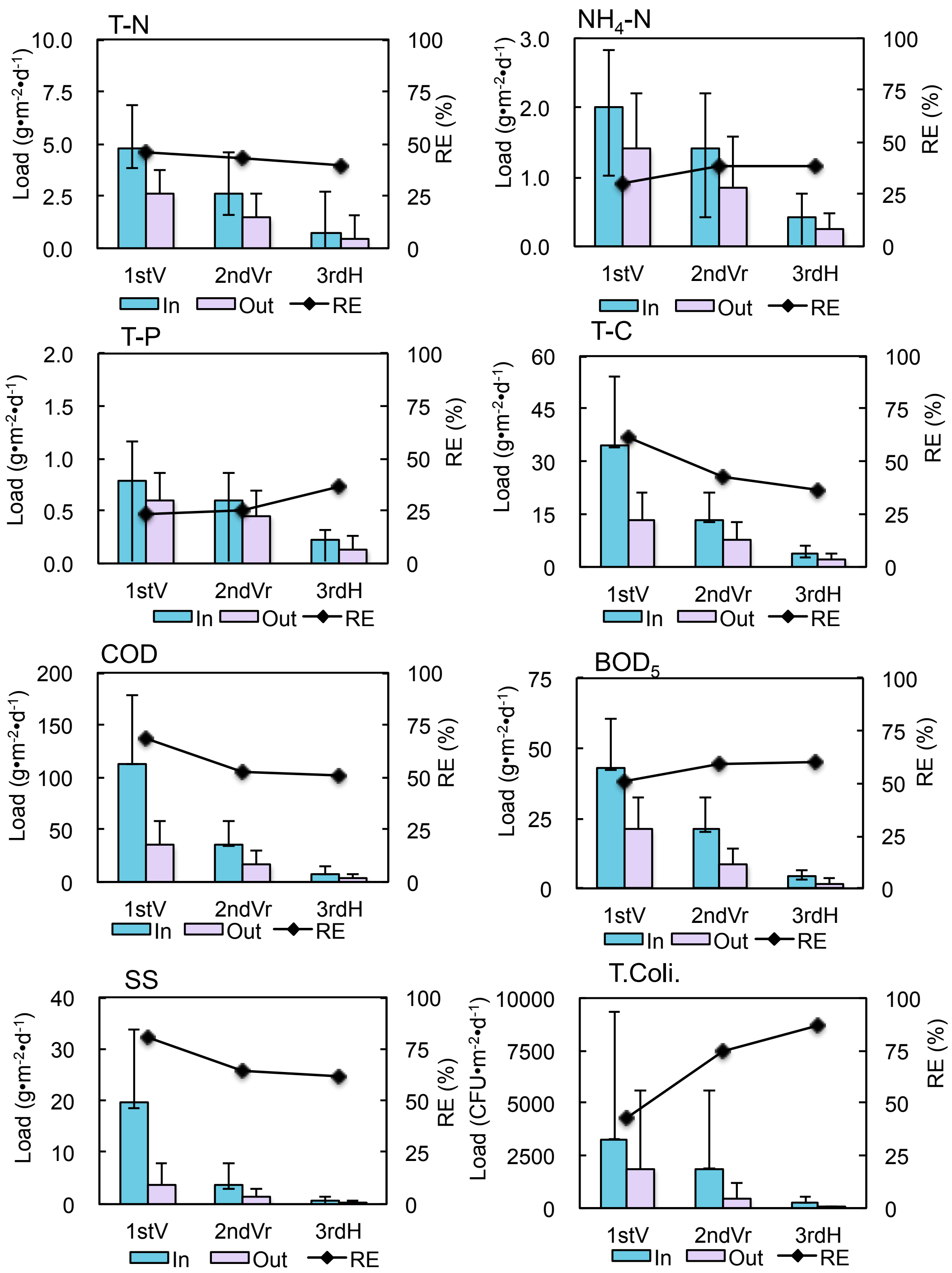

Fig. 3-10 Pollutant's inflow (in) load and outflow (out) load, as well as removal efficiency (RE) at each bed of Dairy-S.

3.3.4 Yearly removal performance

Fig. 3-11 shows annual inflow and final outflow load levels of T-N, NH_4-N, T-P, T-C, COD, BOD_5, SS, and T.Coli. in form of box-and-whisker diagrams after nine years of operation, along with annual removal efficiency of Dariy-S.

The T-N and NH_4-N removal efficiencies were high in the first year and presented increased tendency in the following years except in the second year and the eighth year. In the winter of 8^{th} year (2014), surface covering organic matters were removed partly from the 1^{st} V and 2^{nd} Vr, hence the removal of TN, NH_4-N, TC, COD, and BOD_5 increased in the ninth year compared with eighth year. Dairy-S's removal efficiency of COD, and BOD_5 was around 90% in the first year after construction, and maintained high value from the beginning of operation.

Dairy-S's inflow load of T-P was small and stayed at a consistent level during these four years, but T-P's removal efficiency presented a decreasing tendency yearly. This could be due to saturation of the sand's adsorption capacity over time. The whole system also performed well in the removal of SS and T.Coli..

Overall, the hybrid CW system had high and stable pollutant removal efficiency year by year.

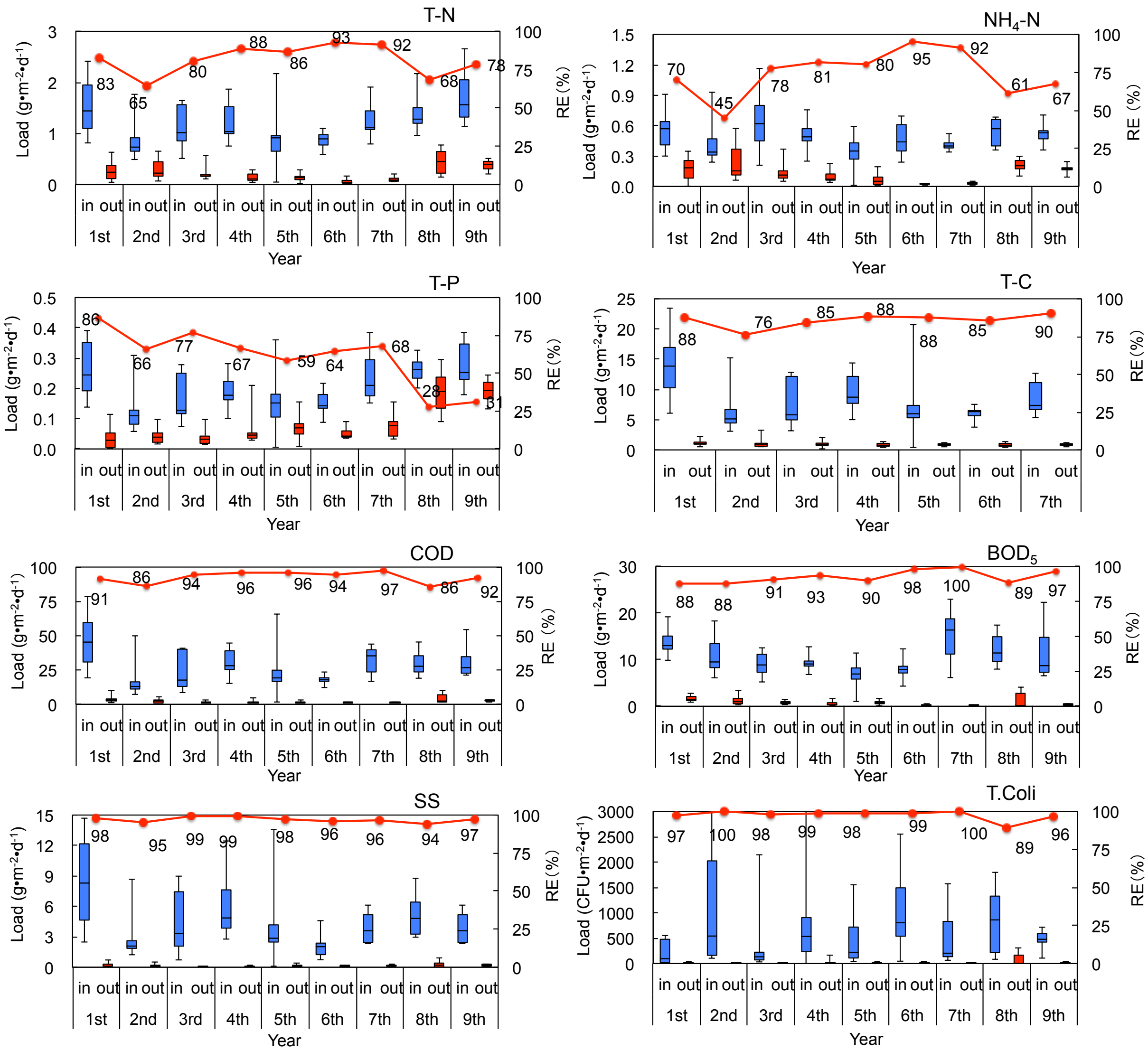

Fig. 3-11 Yearly pollutant inflow load (in) and outflow load (out), and the yearly removal efficiency (RE) of Dairy-S.

3.3.5 Pollutant removal efficiency in cold and warm period

Fig. 3-12 shows the Dairy S's removal efficiency at each bed during the cold period and warm period.

In the 1st V bed, removal efficiency of NH_4-N and COD was a little higher in warm than in cold periods, for other pollutants, they presented similar removal efficiency except T-P. While in the 2nd Vr, T-N, NH_4-N, T-P, T-C and COD had higher removal efficiency in warm period compared with in cold period, while BOD_5, SS, and T.Coli. had high and similar removal efficiency in cold and warm period. In the 3rd H, T-C, COD and BOD_5 had relatively high removal efficiency in warm period than in cold period, while for other pollutants, there were no obvious difference. Totally, the whole system presented high and stable removal efficiency in both cold and warm period for all pollutants removal at each bed.

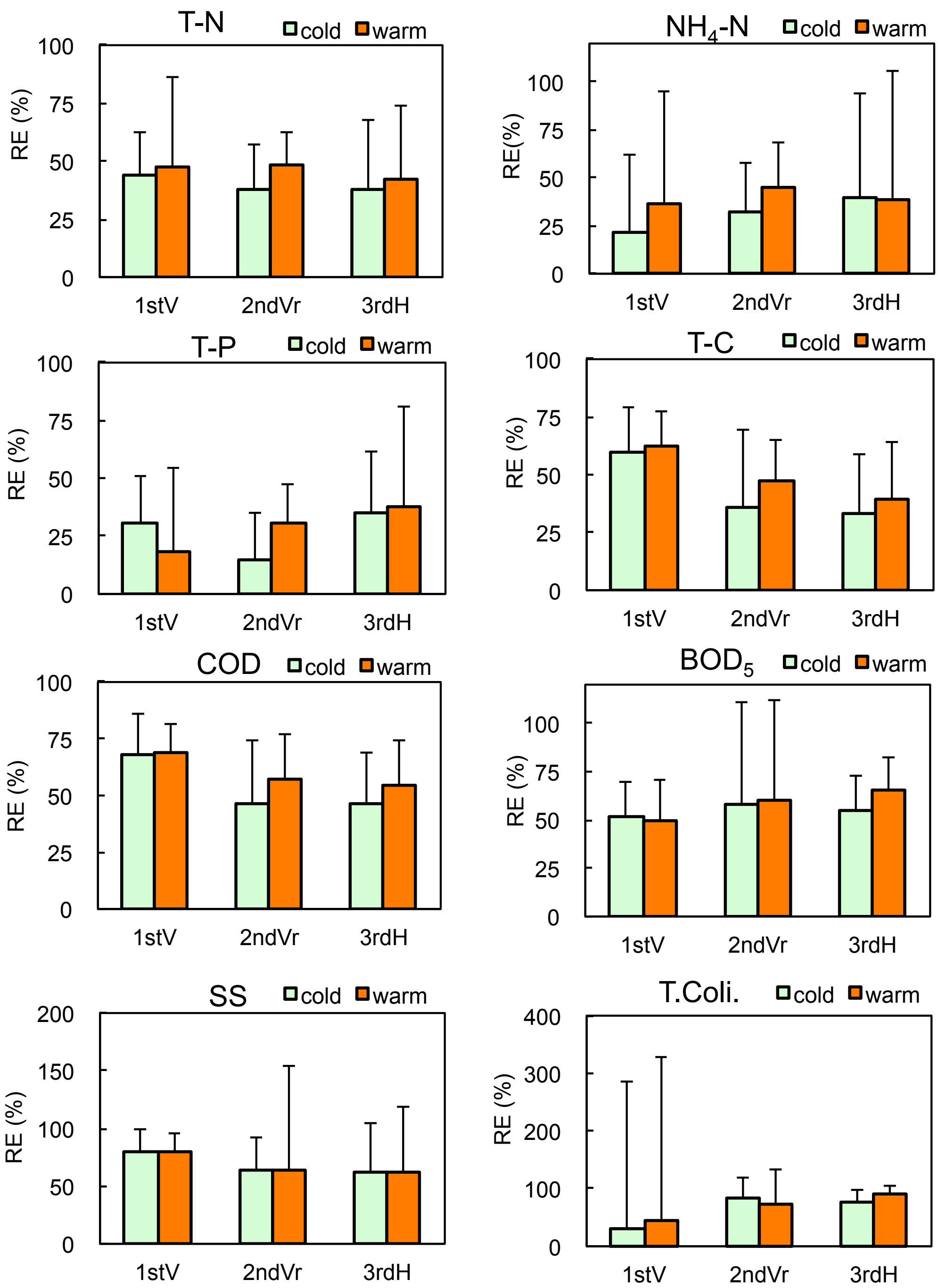

Fig. 3-12 Pollutant's removal efficiency (RE) in cold and warm period at each bed of Dairy-S.

3.5 Discussions

3.5.1 Comparison of the performances of 3 hybrid systems

In the literatures, it generally shows that the overall purification efficiency for T-N was 83% (Molle *et al*., 2008), 79 ~ 86% for well functioning systems (Obarska-Pempkowiak, 2003), 64~79% (Kantawanichkul *et al*., 2003) and 61% (Vymazal, 2005), which is in the same range compared with our systems. Mean while, in our hybrid system, they received high content wastewater, which was extremely high compared with domestic sewage (T-N less than 100 mg•L^{-1}) (Vymazal and Kröpfelová, 2008; Molle *et al*., 2008)

The comparison of pollutant's inflow load, outflow load, and removal efficiency of three hybrid subsurface flow CWs was shown in Fig. 3-13. Totally, Piggery-O received highest inflow T-N load of 11.2 ± 7.5 g•m^{-2}•d^{-1}, and Dairy-S presented highest T-N removal efficiency. The total removal efficiency was around 71 ~ 82%. Compared with other researches (Vymazal, 2013; Borin *et al*., 2013; Lee *et al*., 2004), these hybrid subsurface flow CWs performed high removal efficiency for T-N removal. All systems received high COD load, especially in Diary-G, where the inflow load was 124.0 ± 58.5 g•m^{-2}•d^{-1}. All of these three systems performed well for COD and BOD_5 removal since the beginning of construction.

For T-P, these three systems performed removal rates of 0.9 ± 0.8, 0.4 ± 0.2, and 0.1 ± 0.1 g•m^{-2}•d^{-1} of Piggery-O, Dairy-G, and Dairy-S, respectively. The latter findings are similar with findings reported by Vymazal (2013). The different removal efficiency might be attributed to the bed material used in these systems had different P absorption capacity.

As described in the previous sections, the initial milking parlour wastewater contained large amount of Org-N compared with swine wastewater, where swine urine wastewater contained greater amount of NH_4-N. In Piggery-O, the greatly increase of NO_3-N indicated that nitrification happened in this system. For Dairy-G, ammonification occurs and Org-N was decomposed into a large amount of NH_4-N, and then nitrification and denitrification both existed, and the concentration of NH_4-N decreased gradually as well as NO_3-N. After passed through the 5^{th} V in both Piggery-O and Dairy-G, NO_3-N increased because of the nitrification occurred. But for Piggery-O, the T-N didn't decreased after wastewater passed through the 5^{th} V, another H bed might be added to transform NO_3-N into N gaseous if it is necessary. Overall, there was no significant difference of COD and BOD_5 removal efficiency in both warm period and cold period in every system. This could be expected on basis of findings reported by Steinmann *et al*. (2003) and Akratos *et al*. (2007), which indicated that organic matter removal was not significantly affected by temperature.

The efficiency of T-P removal was similar during both warm and cold periods. The finding is consistent with a previous research, which indicates temperature does not significantly influence T-P removal (Kadlec and Reddy, 2001). Wetlands do not provide the direct metabolic pathway to remove P. Wetlands use physical, chemical, and biological means to reduce P, adsorption of P through soil media is mostly used. In this study, supersol (lightweight porous recycled glass) and pumiceous gravel was used as effective bed material for P adsorption, this is temperature independent. A similar conclusion was also observed in the study reported by Sharma *et al*., (2013), who studied the seasonal efficiency of a hybrid subsurface flow constructed wetland system. And in his research, he also gave several examples that indicated the P removal had no difference between cold and warm seasons.

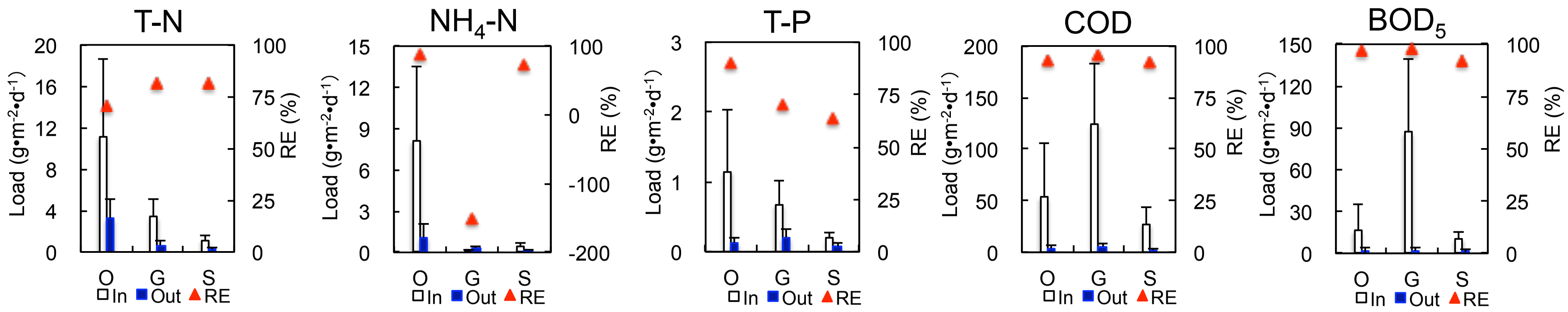

Fig. 3-13 Comparison of pollutant's inflow load (in), outflow load (out), and removal efficiency (RE) of three hybrid subsurface flow CWs.

3.5.2 Correlations between parameters and pollutant's removal efficiency

Pearson correlation coefficients were estimated by using SPSS 19.0 to evaluate correlations between environmental parameters and removal efficiency.

Table 3-10 shows correlations between pollutant's removal efficiency and environmental parameters such as COD/T-N ratio, DO, T and pollutant concentration in Piggery-O.

In Piggery-O, the removal efficiency of N, P, T-C, COD, BOD_5, and T.Coli. might had significant relationship with SS concentration at different bed stage, either negative or positive, either at $p < 0.05$ level or $p < 0.05$. This might be explained that a large amount of pollutants attached or contained to the particle existed in the wastewater, and then was removed as SS.

The amount of DO might have had significant positive effect on NH_4-N removal in the 3rd V of Piggery O, while the negative relationship presented in 4th H. Some studies indicated that effluent DO from a wetland is not necessarily a good indicator of the wetland matrix's aerobic/anaerobic conditions (Vymazal, 2008). There was a significant negative correlation between COD/T-N ratio and NH_4-N removal efficiency in this system. This could be attributed to the fact that a high COD load would likely consume oxygen for degradation, thereby affecting nitrification to some extent (Tanner and Kadlec, 2003).

Table 3-11 shows correlations between pollutant's removal efficiency and environmental parameters such as COD/T-N, DO, T and pollutant concentration in

Dairy-G.

In Dairy-G, T-N removal was firstly related with Org-N, which was predominated in the original inflow, thus the DO had a negative removal relationship with N removal, because Org-N was decomposed firstly and nitrification was inhibited or occurred in the next step. Hence the DO had a negative relationship with T-N removal efficiency. COD/T-N ratio varied greatly in this system, with a range between 11 in the 5^{th} V to 166 in the 3^{rd} V. COD/T-N had a positive relationship with T-N removal in the 4^{th} H, this might be connected with the denirification existed in this bed, where organic compound is necessary as electron donors for denitrfiication.

In Dairy-S, similar relationship was observed between COD/T-N ratio and T-N removal efficiency, while the COD/T-N varied from 11 to 23. More details were shown in Table 3-12.

Table 3-10 Correlations (Pearson coefficient) between environmental parameters, pollutant concentration (C) and pollutant removal efficiency (R) at each bed of Piggery-O.

Piggery-O		C (T-N)	C (NH_4-N)	C (T-P)	C (COD)	C (BOD_5)	C (SS)	C (T.Coli.)	C (T-C)	C (PO_4-P)	C (Org-P)	C (Org-N)	pH	EC	DO	T	ORP	COD/ T-N
1st Vr	R (T-N)													-0.63**				
	R (NH_4-N)													-0.41**				-0.30*
	R (T-P)			0.27*							0.37**			-0.70**				
	R (COD)			0.28*							0.31*			-0.67**				
	R (BOD_5)										0.26*			-0.68**				
	R (SS)																0.41*	
	R (T.Coli.)																	
	R (T-C)									-0.33*	0.34*			-0.67**				
	R (PO_4-P)												0.33**	-0.59**				
	R (Org-P)			0.33**							0.43**			-0.53**				
	R (Org-N)													-0.71**				
2nd Vr	R (T-N)						0.38*											
	R (NH_4-N)							-0.50**					-0.29*				0.31*	-0.34*
	R (T-P)	0.34**	0.27*	0.54**	0.32*	0.34*	0.51**			0.34**	0.61**							0.30*
	R (COD)			0.36**		0.27*	0.61**				0.53**							0.38**
	R (BOD_5)						0.42*				0.31*							
	R (SS)						0.46**								0.41*	-0.35*		
	R (T.Coli.)																	
	R (T-C)						0.44*	-0.39*			0.42**							0.35*
	R (PO_4-P)	0.47**	0.34**	0.55**	0.40**	0.39**	0.34*		0.40**	0.53**	0.42**			0.38**		-0.32*		
	R (Org-P)						0.47**								0.30*			
	R (Org-N)											0.39**			-0.34*			0.32*
3rd V	R (T-N)										0.41**							
	R (NH_4-N)	-0.30*	-0.38**	-0.28*	-0.35**	-0.34**			-0.38**				-0.48**	-0.34**	0.43**	-0.32*	0.35**	-0.43**
	R (T-P)	0.26*	0.28*	0.32*	0.26*			0.34*			0.51**							
	R (COD)										0.31*							
	R (BOD_5)										0.28*		0.40**		-0.34**		-0.42**	0.30*
	R (SS)											-0.41**					0.41*	
	R (T.Coli.)															-0.37*		
	R (T-C)	-0.36*	-0.38**		-0.37*	-0.36*			-0.39**				-0.30*	-0.37*	0.49**		0.30*	-0.39**
	R (PO_4-P)																-0.40**	
	R (Org-P)										0.31*	-0.27*			0.33*	-0.47**	0.39**	
	R (Org-N)																	

Piggery-O		C (T-N)	C (NH_4-N)	C (T-P)	C (COD)	C (BOD_5)	C (SS)	C (T.Coli.)	C (T-C)	C (PO_4-P)	C (Org-P)	C (Org-N)	pH	EC	DO	T	ORP	COD/ T-N
4[th] H	R (T-N)						0.36*						0.27*				-0.37**	
	R (NH_4-N)														-0.34**	0.48**		
	R (T-P)	0.36**	0.42**	0.39**	0.41**	0.38**	0.32*	0.41**	0.44**	0.33*	0.39**			0.34**	-0.46**			0.50**
	R (COD)		0.27*	0.39**	0.26*		0.60**	0.44**		0.30*	0.39**				-0.38**	0.26*		0.42**
	R (BOD_5)									0.26*			-0.27*					
	R (SS)			0.57**		0.33*	0.66**	0.33*		0.35*	0.58**	0.44**		0.39*	-0.57**	0.34*		
	R (T.Coli.)																0.33*	
	R (T-C)																	
	R (PO_4-P)	0.33**	0.36**	0.27*	0.34**	0.34**			0.32*	0.35**				0.32*				0.27*
	R (Org-P)														-0.37**			
	R (Org-N)	-0.34*	-0.45**	-0.40**	-0.45**	-0.47**			-0.46**	-0.43**	-0.32*			-0.45**	0.31*			-0.34*
5[th] V	R (T-N)	0.42**	0.45**	0.27*	0.44**	0.42**			0.42**		0.30*		0.42**	0.40**				0.45**
	R (NH_4-N)	-0.56**	-0.66**		-0.54**	-0.50**			-0.56**				-0.76**	-0.54**			0.31*	-0.52**
	R (T-P)			0.38**			0.46**			0.27*	0.41**							
	R (COD)			0.28*							0.26*	0.25*			-0.34**			
	R (BOD_5)			0.36**						0.34**	0.29*	0.30*			-0.42**	0.30*		
	R (SS)						0.33*					-0.39*						
	R (T.Coli.)						-0.40*						-0.49**				0.57**	-0.53**
	R (T-C)	-0.36*	-0.38**										-0.43**	-0.36*		0.30*		
	R (PO_4-P)						0.32*											0.29*
	R (Org-P)			0.27*							0.30*							
	R (Org-N)																	

Notes: EC, electrical conductivity. DO, dissolved oxygen ($mg \bullet L^{-1}$). T, temperature (°C). ORP, oxidation-reduction potential.

** significant at $p < 0.01$ level;

* significant at $p < 0.05$ level.

Table 3-11 Correlations (Pearson coefficient) between environmental parameters, pollutant concentration (C) and pollutant removal efficiency (R) at each bed of Dairy-G.

Dairy-G		C (T-N)	C (NH_4-N)	C (T-P)	C (COD)	C (BOD_5)	C (SS)	C (T.Coli.)	C (T-C)	C (PO_4-P)	C (Org-P)	C (Org-N)	pH	EC	DO	T	ORP	COD/ T-N
1st Vr	R (T-N)	0.41*				0.42*						0.40*	0.56**	-0.41*			0.41*	-0.63**
	R (NH_4-N)		0.65**							0.42*								
	R (T-P)	0.64**		0.54**	0.57**	0.62**	0.54**			0.52**	0.48**	0.64**	0.46*					-0.44*
	R (COD)					0.42*	0.43*						0.53**					-0.40*
	R (BOD_5)	0.59**		0.54**	0.52*	0.58**	0.50*			0.71**		0.59**			-0.48*			-0.48*
	R (SS)												0.44*					
	R (T.Coli.)														0.49*			
	R (T-C)													-0.71**				-0.57*
	R (PO_4-P)	0.40*				0.47*	0.42*			0.43*		0.39*	0.38*					-0.46*
	R (Org-P)			0.43*							0.70**							
	R (Org-N)												0.54**					-0.57**
2nd V	R (T-N)		0.39*												-0.55**		-0.46*	
	R (NH_4-N)	-0.40*		-0.50**	-0.56**				-0.68**	-0.61**		-0.41*		-0.40*				-0.47**
	R (T-P)										0.68**							
	R (COD)	0.53**		0.47**	0.48**				0.52*	0.51**	-0.39*	0.52**		0.52**	-0.44*		-0.51**	
	R (BOD_5)							0.62**										
	R (SS)	0.49*		0.57**	0.45*		0.47*		0.77*	0.49*				0.52*		-0.62**		
	R (T.Coli.)		-0.47*															
	R (T-C)	0.76**	0.65**	0.68**	0.64**			0.95*	0.62*	0.75**		0.67**		0.84**			-0.66*	
	R (PO_4-P)							-0.52*										
	R (Org-P)	-0.38*			-0.39*					-0.47**	0.51**	-0.37*		-0.46*				
	R (Org-N)		0.52**	0.48**					0.77**	0.55**				0.49**			-0.52**	
3rd V	R (T-N)	0.48**			0.41*							0.50**						
	R (NH_4-N)	-0.39*			-0.42*	-0.54**	-0.56**					-0.51**		0.60**				
	R (T-P)		0.41*						0.59*					0.44*	0.39*			
	R (COD)			0.49**				0.56*	0.55*					0.43*	0.51**			
	R (BOD_5)							0.48*										
	R (SS)							0.51*										
	R (T.Coli.)	-0.53*										-0.60**						
	R (T-C)	0.62*		0.53*	0.65**	0.75*			0.62*					0.56*	0.55*			
	R (PO_4-P)		0.53**	0.42*	0.43*			0.46*	0.75**	0.45*				0.41*	0.40*			
	R (Org-P)										0.45*		-0.43*					
	R (Org-N)	0.50**			0.54**				0.56*	0.47**		0.49**						

Dairy-G		C (T-N)	C (NH_4-N)	C (T-P)	C (COD)	C (BOD_5)	C (SS)	C (T.Coli.)	C (T-C)	C (PO_4-P)	C (Org-P)	C (Org-N)	pH	EC	DO	T	ORP	COD/ T-N
4th H	R (T-N)	-0.41^{*}		-0.46^{**}						-0.41^{*}		-0.50^{**}		-0.57^{**}				0.64^{**}
	R (NH_4-N)							-0.65^{**}				-0.60^{**}						
	R (T-P)	-0.60^{**}		-0.59^{**}		-0.45^{*}	-0.46^{*}			-0.50^{**}		-0.63^{**}		-0.54^{**}	0.39^{*}			
	R (COD)							0.52^{*}										
	R (BOD_5)		-0.57^{**}							-0.48^{*}					-0.48^{*}			
	R (SS)		-0.55^{**}	-0.46^{*}														
	R (T.Coli.)											0.51^{*}		0.58^{**}	-0.50^{*}			
	R (T-C)							-0.99^{**}										0.59^{*}
	R (PO_4-P)			-0.40^{*}								-0.40^{*}		-0.41^{*}	0.49^{*}			
	R (Org-P)	-0.46^{*}		-0.41^{*}	-0.40^{*}	-0.60^{**}	-0.50^{*}	-0.67^{**}				-0.65^{**}		-0.44^{*}				
	R (Org-N)																	0.43^{*}
5th V	R (T-N)																	
	R (NH_4-N)	-0.58^{**}	-0.52^{**}	-0.55^{**}	-0.74^{**}	-0.76^{**}	-0.76^{**}	0.60^{**}	-0.62^{*}	-0.43^{*}	-0.49^{**}	-0.51^{**}		-0.49^{**}			0.63^{**}	
	R (T-P)														0.39^{*}		0.39^{*}	
	R (COD)					0.61^{**}	0.68^{**}						0.47^{*}				-0.46^{*}	
	R (BOD_5)					0.46^{*}	0.52^{*}				0.42^{*}							
	R (SS)		0.48^{*}				0.46^{*}			0.58^{**}					-0.54^{*}			
	R (T.Coli.)																	
	R (T-C)																-0.60^{*}	
	R (PO_4-P)							0.48^{*}	0.63^{**}						-0.42^{*}	-0.46^{*}		
	R (Org-P)					-0.48^{*}	-0.44^{*}										0.42^{*}	
	R (Org-N)							-0.76^{**}										

Notes: EC, electrical conductivity. DO, dissolved oxygen ($mg \bullet L^{-1}$). T, temperature (°C). ORP, oxidation-reduction potential.

** significant at $p < 0.01$ level;

* significant at $p < 0.05$ level.

Table 3-12 Correlations (Pearson coefficient) between environmental parameters, pollutant concentration (C) and pollutant removal efficiency (R) at each bed of Dairy-S.

Dairy-S		C (T-N)	C (NH_4-N)	C (T-P)	C (COD)	C (BOD_5)	C (SS)	C (T.Coli.)	C (T-C)	C (PO_4-P)	C (Org-P)	C (Org-N)	pH	EC	DO	T	ORP	COD/ T-N
	R (T-N)	0.37**	0.35**	0.35**	0.29**		0.26*		0.31**	0.34**	0.22*	0.26*						
	R (NH_4-N)		0.33**												-0.23*	0.43*		-0.36**
	R (T-P)	0.57**	0.44**	0.52**	0.47**	0.27*	0.44**		0.50**	0.51**	0.28**	0.44**						
	R (COD)					-0.36**												
	R (BOD_5)										0.34**						-0.29*	
1st Vr	R (SS)					-0.31**					0.34**	0.26*	-0.24*					
	R (T.Coli.)	-0.34**	-0.50**	-0.35**	-0.38**	-0.24*	-0.33**		-0.43**	-0.38**					0.25*			-0.29**
	R (T-C)					-0.43**					0.25*							
	R (PO_4-P)	0.48**	0.43**	0.45**	0.39**	0.27*	0.35**		0.41**	0.45**	0.22*	0.35**						
	R (Org-P)	0.26*					0.23*				0.30**	0.29**						
	R (Org-N)	0.45**	0.22*	0.35**	0.47**		0.45**		0.52**	0.39**		0.40**	-0.33**				0.29*	0.34**
	R (T-N)		-0.37**		-0.33**	-0.25*	-0.29**		-0.33**					-0.28*				-0.34**
	R (NH_4-N)	-0.50**	-0.49**		-0.46**	-0.46**	-0.39**		-0.52**			-0.29**		-0.48**		0.58**	0.25*	-0.25*
	R (T-P)		0.21*			0.31**	0.23*	0.25*							-0.27*	0.49*		
	R (COD)			0.23*						0.24*								0.30**
	R (BOD_5)																	0.22*
2nd Vr	R (SS)	0.24*				0.26*	0.26*											
	R (T.Coli.)							0.29**								0.45*		
	R (T-C)			0.28*						0.26*	0.23*	0.32**			-0.35**			
	R (PO_4-P)	0.24*	0.25*		0.21*	0.31**	0.25*	0.28*	0.25*	0.21*					-0.27*	0.53**		
	R (Org-P)									-0.22*		-0.32**		-0.37**				
	R (Org-N)							0.24*				0.31**			-0.44**			
	R (T-N)		0.25*						0.31**	0.23*			-0.37**	-0.26*				
	R (NH_4-N)	0.35**	0.43**	0.27*	0.29**				0.46**	0.30**			-0.35**	-0.23*	0.23*	-0.65**	0.29*	
	R (T-P)		0.34**		0.27*	0.37**			0.38**				-0.40**	-0.28**	0.37**	-0.50*	0.25*	
	R (COD)		0.25*	0.35**						0.40**			-0.24*			-0.45*	0.26*	
	R (BOD_5)			0.24*		-0.28**				0.27*								
3rd V	R (SS)						0.28**	-0.30**						-0.28**	0.26*			-0.24*
	R (T.Coli.)				-0.22*		-0.23*		-0.24*			-0.23*						-0.31**
	R (T-C)	0.47**	0.48**	0.51**	0.48**	0.33**	0.33**		0.54**	0.48**	0.44**	0.24*	-0.42**		0.25*			0.34**
	R (PO_4-P)		0.34**		0.27*	0.38**			0.38**				-0.37**	-0.26*	0.35**	-0.47*	0.23*	
	R (Org-P)												-0.37**	-0.33**	0.28*	-0.58**	0.26*	
	R (Org-N)				-0.23*	-0.30**												

Notes: EC, electrical conductivity. DO, dissolved oxygen ($mg \cdot L^{-1}$). T, temperature (°C). ORP, oxidation-reduction potential.

** significant at $p < 0.01$ level;

* significant at $p < 0.05$ level.

The COD/T-N ratio of Piggery-O was lower than for milking parlor wastewater, thus the carbon deficiency maybe occurring in this system and carbon is becoming a limiting agent of denitrification. Fig. 3-14 shows a relationship between concentrations of NH_4-N, NO_3-N, and T-C of Piggery-O. When organic compounds are insufficient, denitrifcation may be limited. High concentration of NO_3-N started to be seen at low T-C. This may have happened because the denitrifying bacteria did not have enough carbon to denitrify all NO_3-N that were transformed from NH_4-N. Therefore NH_4-N could be nitrified to N/O_3-N, but NO_3-N stayed in wastewater, without denitrification.

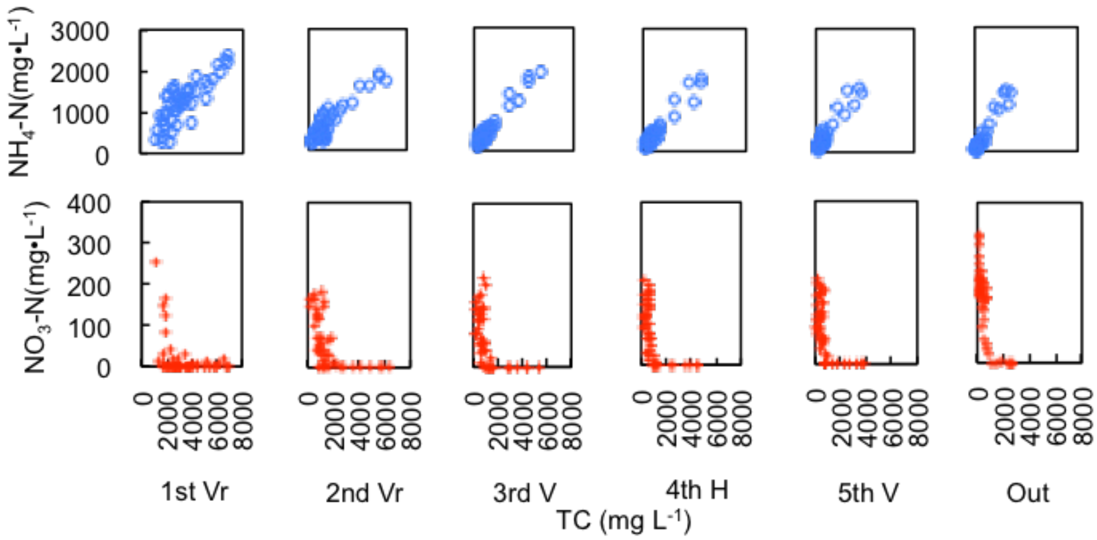

Fig. 3-14 Relationship between nitrogen concentration and T-C concentration in inflow of each bed and final outflow of Piggery-O.

Chapter 4 N balance assessment

4.1 Study site

With the purpose of a better understanding of N transform cycle in hybrid system, bed material samples were taken to analysis N stored there.

In Piggery-O, August 2014, 3 samples were collected in the midrange of 1st Vr uniformly, and 2 samples were collected in the center of 2nd Vr, 3rd V, 4th H, and 5th V. For Dairy-S, 2 samples were collected in the 1st V and 2nd Vr, and another 6 samples were collected in 3rd H bed in June 2014.

4.2 Sampling and analysis

Bed materials were sampled by use of core sampler in Piggery-O and Dairy-S. The size of cylindrical core sampler was 25 cm in height, with diameter of 10 cm.

After sampling, samples were firstly divided into different small samples on basis of bed filter materials in lab. The surface layer including covering organic matters and Supersol or ALC was also considered as a part of bed samples.

Later, samples were dried at 60 °C and the weight was recorded. After this, samples were smashed into powder, and the N content was analyzed by detecting the nitrogen composition percentage of the sample by use of Vario MAX CN analyzer. The amount of N attached on the surface of gravel was analyzed by soaking the gravel into the water for abundant length of time, and N content analysis methods is same with section 2.4.

4.3 Calculation

N mass stored in each bed materials is calculated as:

$$m_{(N)} = m_{(T)} \times P_{(N)}$$

Where, $m_{(T)}$ is total mass of each kind of bed material, $P_{(N)}$ means N percentage of each kind of bed material.

4.4 Results and discussions

As shown in table 4-1, N mass stored in each material layer at each bed of Piggery-O was indicated, and the absorption ability of each material was also shown here. Average N absorptivity of the bed materials was 5.16 kg N m^{-3} for organic matter, 1.31 kg N m^{-3} for ALC, and 0.86 kg N m^{-3} for porous pumice gravel, respectively.

As shown in Fig. 4-1, N balance analysis indicated that the surface organic matter (Org-M) layer contained 36% of T-N that stored in beds, ALC took account of 22 % T-N, while the T-N stored in porous pumice gravel was 42.0 %.

Fig. 4-2 indicated the amount of N contained at each bed of Piggery-O. The 1st Vr and 2nd Vr stored large amount of N compared with the other beds.

Totally, the amount of N contained (stored) in bed material was 1,358 kg, took account of 9% of the total N removed (15,579 kg) by whole Piggery-O system. The transformation of received N at each bed was shown in Fig. 4-3, except N loaded out to the next bed stage, N stored in bed layer was not large, and a large amount of N was converted into N gaseous, and then released into atmosphere in form of N gaseous, which indicated that denitrification was an important process for N removal in this hybrid CWs treating piggery wastewater.

Table 4-1 N composition, N contained mass, and N absorption ability of each bed material layer of Piggery-O.

Piggery-O	Bed material layer	N composition (%)	Total mass (kg)	Mass of contained N (kg)	N absorption ability ($kg•m^{-3}$)
1^{st} Vr	Org-M	1.14	11,191	128	3.20
	ALC	0.43	16,356	70	1.67
	PG (L)	0.16	49,706	81	0.85
	ALC	0.24	93,152	223	0.95
2^{nd} Vr	Org-M	1.65	10,419	172	5.70
	PG (M)	0.27	49,092	133	1.22
	PG (L)	0.20	70,200	139	0.92
3^{rd} V	Org-M	1.42	12,337	175	6.57
	PG (S)	0.16	31,352	51	1.22
	PG (M)	0.11	30,848	34	0.67
4^{th} H	PG (M)	0.11	49,621	56	0.71
	PG (S)	0.13	42,285	57	1.07
5^{th} V	Org-M	0.12	15,184	19	1.01
	PG (S)	0.10	1,332	1	0.46
	PG (L)	0.10	16,906	18	0.65

Note: Org-M: organic matter. ALC: autoclaved lightweight aerated concrete. PG: pumice gravel (L: large size; M: middle size; S: small size).

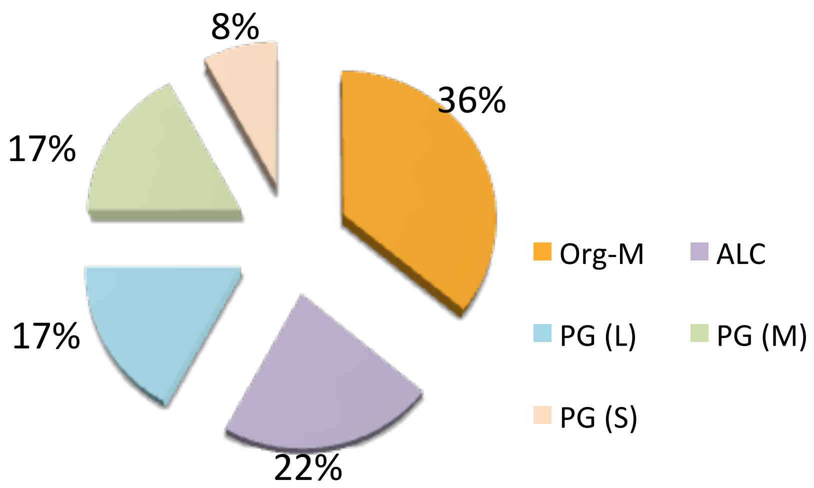

Fig. 4-1 Percentage of N contained at each bed material layer against total N stored in all bed (Piggery-O). Org-M: organic matter. ALC: autoclaved lightweight aerated concrete. PG: pumice gravel (L: large size; M: middle size; S: small size.

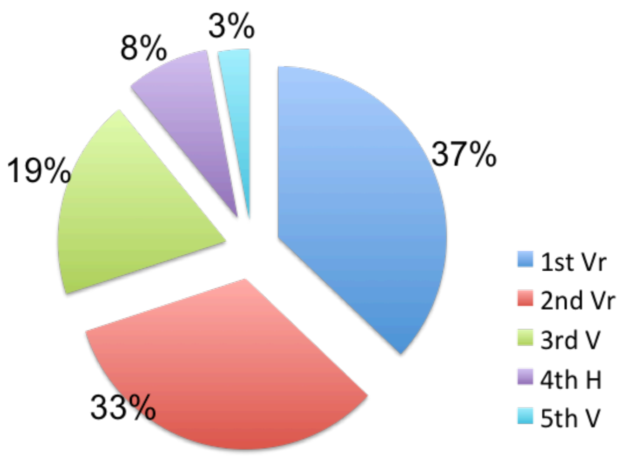

Fig. 4-2 Percentage of N contained at each bed against total N stored in all bed (Piggery-O).

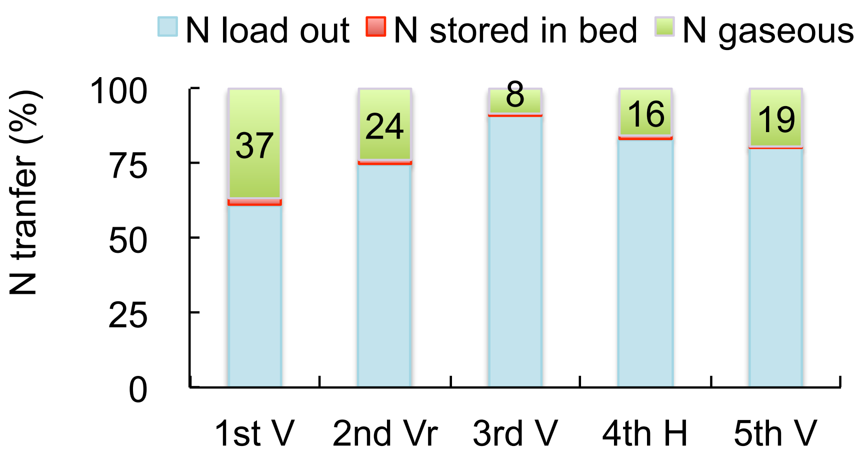

Fig. 4-3 N transformation at each bed of Piggery-O.

N mass stored in each material layer at each bed of Dairy-S was shown in table 4-2. Average N absorptivity of each material layer was 6.51 kg N m^{-3} for organic matter, 0.14 kg N m^{-3} for gravel, 3.19 kg N m^{-3} for clinker ash, and 0.20 kg N m^{-3} for sand.

Fig. 4-4 indicated that in Dairy-S, Org-M layer stored a large amount of N, nearly 87% of N that stored in bed was contained in this layer. Among the Org-M layers of 3 beds, the Org-M layer of 1st V contained 690 kg of N stored in bed, nearly 80% (Fig. 4-5). This indicated the surface Org-M layer might be an un-negligible part for N removal in hybrid system that treats milking parlor wastewater.

N transformations at each bed of Dairy-S was shown in Fig. 4-6, in the 1st V, 35% of received N was stored in bed, while 15% was released into atmosphere. While in the 2nd Vr and 3rd H, 35% and 39% of received N was released into atmosphere. This indicated that denitrification occurred obviously in Dairy-S since a large percentage of N gaseous released. Totally, the bed material stored nearly 48% of N removed by Dairy-S.

Table 4-2 Total mass, N contained mass, and N absorption ability of each bed materials of Dairy-S.

Dairy-S	Bed material layer	N composition		Total mass	Mass of contained N	N absorption ability
		(%)	(mg•g^{-1})	(kg)	(kg)	(kg•m^{-3})
1st V	Org-M＋Supersol	3.37	-	20,490	690	17.18
	Gravel	-	0.08	73,422	6	0.14
	Gravel	-	0.05	68,065	3	0.08
2nd Vr	Org-M	0.45	-	6,473	29	1.71
	Clinker ash	0.38	-	12,963	49	3.19
	Gravel	-	0.11	143,712	16	0.20
3th H	Org-M＋ALC	0.07	-	53,652	40	0.64
	Washed sand	0.05	-	79,575	36	0.20

Note: Org-M: organic matter. ALC: autoclaved lightweight aerated concrete

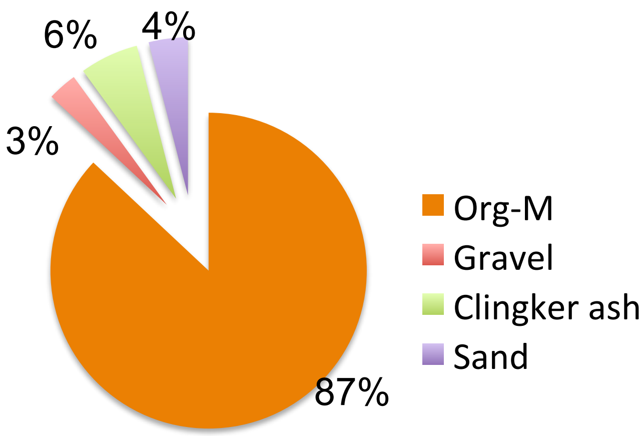

Fig. 4-4 Percentage of N contained at each bed material layer against total N stored in all bed (Dairy-S) Org-M: organic matter.

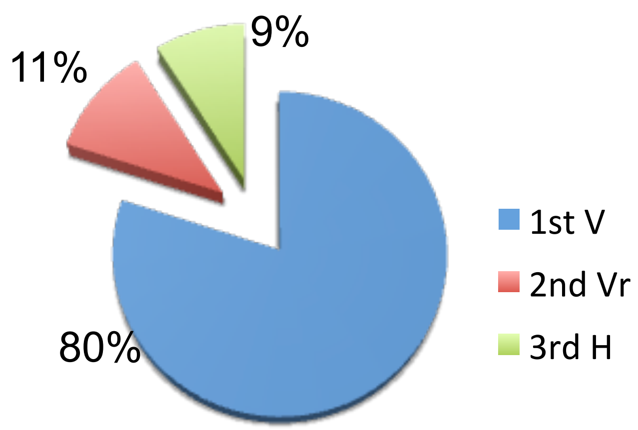

Fig. 4-5 Percentage of N contained at each bed against total N stored in all bed (Dairy-S).

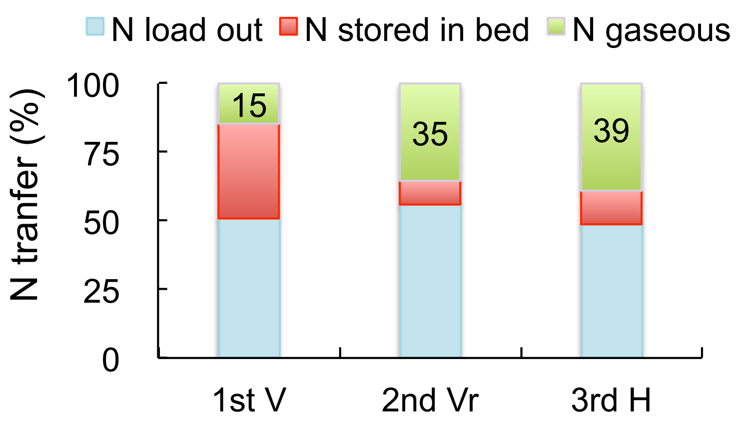

Fig. 4-6 N transformation at each bed of Dairy-S.

Chapter 5 Conclusions

After years of operation, all three systems of Piggery-O, Dairy-G, and Dairy-S performed high treatment efficiency for pollutant removal and wastewater purification. The purification efficiency of these systems varied because of the system design and inflow wastewater concentrations were different. Totally, all systems performed high COD and BOD_5 purification efficiency even they received high strength wastewater. The purification efficiency of T-N was 72 ± 9%, 85 ± 7%, and 86 ± 12 of Piggery-O, Dairy-G, and Dairy-S, respectively.

For T-P, the Piggery-O had highest purification efficiency of 91 ± 6% compared with 75± 9% and 75 ± 28% of Dairy-G and Dairy-S. The different purification efficiency could be attributed to the material used in the bed. The porous pumice gravel used in the Piggery-O system presented high P absorption ability compared with others.

The yearly pollutant removal of each system presented stable and high removal efficiency for COD and BOD_5. T-N removal in Piggery-O performed an increasing tendency year after year since construction, meanwhile, the T-P removal maintained high removal from the beginning. In contrast, T-N removal in Dairy-S performed high removal efficiency from the first year of construction, but for T-P, there was a decreased removal tendency after years of operation, this might be explained by the saturated P absorption of bed material used in Dairy-S. Totally, pollutant removal efficiency of these three hybrid systems was high compared with other researches.

N removal mechanisms varied in different hybrid systems, which was concerned with the system's conditions such as DO and N composition. In Piggery-O, the original inflow wastewater contained large amount of NH_4-N, as a result, NH_4-N was converted into NO_3-N by nitrification, and then into N_2 by denitrification. While the organic matter was limited, NO_3-N was accumulated in the 5th bed. But in Dairy-G, the original inflow wastewater contained large amount of Org-N. Hence the Org-N decomposition occurred firstly, and the nitrification was inhibited in the first step, thus the NH_4-N firstly increased and then decreased according to nitrification.

The N transformation of received N at each bed of hybrid systems also performed varied style in Piggery-O and Dairy-S. For Piggery-O, N stored in bed layer was not large, and nearly 91% of N removed by whole system was released in form of N gaseous, while only 9% of removed N was stored in the bed, this indicated denitrification was an important process for N removal in Piggery-O. In contrast, in Dairy-S, 48% was removed N was stored in bed material, and 52% was released in to atmosphere. Among which, surface Org-M layer might be an un-negligible part for hybrid system in N removal. Which indicated in Dairy-S, not only denitrifation, but also adsorption of organic matter layer was an important method for N removal.

References

APHA, American Public Health Association, American Water Works Association, Water Environment Federation 1992: Standard Methods: For the Examination of Water and Wastewater, 18th edition. American Public Health Association, Washington DC, USA.

Akratos CS, Tsihrintzis VA 2007: Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. Ecol Eng., 29 (2), 173-191.

Bavor HJ, Schulz TJ 1993: Sustainable suspended solids and nutrient removal in large-scale, solid-matrix, constructed wetland systems. In: Moshiri, G.A. (Ed.), Constructed Wetlands for Water Quality Improvement. Lewis Publishers, Boca Raton, Florida, pp. 219-225.

Borin M, Politeo M, Stefani GD 2013: Performance of a hybrid constructed wetland treating piggery wastewater. Ecol Eng., 51, 229-236.

Brix H, Arias CA, Johansen NH 2002: BOD and nitrogen removal from municipal wastewater in an experimental two-stage vertical flow constructed wetland system with recycling. In: Proceedings of the Eighth International Conference on Wetland Systems for Water Pollution Control, Arusha, Tanzania, 16-19 September, pp. 400-410.

Cooper D, Griffin P, Cooper PF 2008: Factors affecting the longevity of sub-surface horizontal flow systems operating as tertiary treatment for sewage effluent. In: Vymazal, J. (Ed.), Constructed and Natural Wetlands Springer, Dordrecht, Netherlands, pp. 191-198.

Cooper PF 1999: A review of the design and performance of vertical-flow and hybrid reed bed treatment systems. Water Sci Technol., 40 (3), 1-9.

Cooper PF 2001: Nitrification and denitrification in hybrid constructed wetlands systems. In: Vymazal, J. (Ed.), Transformations of Nutrients in Natural and Constructed Wetlands, pp. 257-270. Backhuys Publishers, Leiden, The Netherlands.

Cooper PF, Job GD, Green MB, Shutes RBE 1996: Reed Beds and Constructed Wetlands for Wastewater Treatment. WRc Publications, UK.

Cronk JK 1996: Constructed wetlands to treat wastewater from dairy and swine operation: a review. Agr. Ecosyst Environ., 58, 97-114.

Doosti MR, Kargar R, Sayadi MH 2012: Water treatment using ultrasonic assistance: A review. Proceedings of IAEES, 2(2), 96-110.

Dunne EJ, Culleton N, O'Donovan G, Harrington R, Olsen AE 2005: An integrated constructed wetland to treat contaminants and nutrients from dairy farmyard dirty water. Ecol Eng., 24(3),

219-232.
Dunne EJ, Reddy KR 2005: Phosphorus biogeochemistry of wetlands in agricultural watersheds. In: Dunne EJ, Reddy R, Carton OT, editors. Nutrient management in agricultural watersheds: a wetland solution, pp. 105-119. Wageningen Academic Publishers, The Netherlands.
Faulwetter JL, Gagnon V, Sundberg C, Chazarenc F, Burr MD, Brisson J, Camper AK, Stein OR 2009: Microbial processes influencing performance of treatment wetlands: A review. Ecol Eng., 35, 987-1004.
Fountoulakis M, Terzakis S, Chatzinotas A, Brix H, Kalogerakis N, Manios T 2009: Pilot-scale comparison of constructed wetlands operated under high hydraulic loading rates and attached biofilm reactors for domestic wastewater treatment. Sci Total Environ., 407, 2996-3003.
Gaboutloeloe GK, Chen S, Barber ME, Sto¨ckle CO 2009: Combinations of horizontal and vertical flow constructed wetlands to improve nitrogen removal. Water Air Soil Pollut., 9(3), 279-286.
Garcı´a J, Rousseau D, Caselles-Osorio A, Story A, DePauw N, Vanrolleghem PA 2007: Impact of prior physico-chemical treatment on the clogging process of subsurface-flow constructed wetlands: model-based evaluation. Water Air Soil Pollut., 185 (1), 101-109.
Garcı´a J, Rousseau DPL, Morato J, Lesage E, Matamoros V, Bayona JM 2010: Contaminant removal process in subsurface-flow constructed wetlands: a review. Critical Reviews in Environ Sci Technol., 40 (7), 561-661.
Griffin P, Wilson L, Cooper, D 2008: Changes in the use, operation and design of sub-surface flow constructed wetlands in a major UK water utility. In: Proceedings of the 11th International Conference on Wetland Systems for Water Pollution Control, pp. 419-426, Vikram University and IWA, Indore, India,
Healy MG, Rodgers M, Mulqueen J 2007: Treatment of dairy wastewater using constructed wetlands and intermittent sand filters. Biores Technol., 98, 2268-2281.
Imfeld G, Braeckevelt M, Kuschk P, Richnow HH 2009: Monitoring and assessing processes of organic chemicals removal in constructed wetlands. Chemosphere, 74 (3), 349-362.
Isaacs SH, Henze M 1995: Controlled carbon source addition to alternating nitrification-denitrification wastewater treatment process including biological P removal. Water Res., 29(1), 77-89.
Japanese Industrial Standards Committee 1998: Testing Methods for Industrial Wastewater. JIS K 0102, Japanese Standards Association, Tokyo, Japan.
Kadlec RH 2000: The inadequacy of first-order treatment wetland models. Ecol Eng., 15,

105-119.

Kadlec RH, Knight RL 1996: Treatment wetlands, pp 893. CRC Press, Boca Raton, Florida, USA.

Kadlec RH, Reddy KR 2001: Temperature effects in treatment wetlands. Water Environ. Res., 73(5), 543-557.

Kadlec RH, Wallace SD 2009: Treatment Wetlands. CRC Press, Boca Raton, Florida, USA.

Kantawanichkul S, Somprasert S 2005: Using a compact combined constructed wetland system to treat agricultural wastewater with high nitrogen. Water Sci Technol., 51(9), 47-53.

Kato K, Inoue T, Ietsugu H, Koba T, Sasaki H, Miyaji N, Kitagawa L, Sharma PK, Nagasawa T 2013b: Performance of six multi-stage hybrid wetland systems for treating high-content wastewater in the cold climate of Hokkaido, Japan. Ecol Eng., 51, 256-263.

Kato K, Inoue T, Ietsugu H, Sasaki H, Harada J, Kitagawa K, Sharma PK 2013a: Design and performance of hybrid constructed wetland systems for high-content wastewater treatment in the cold climate of Hokkaido, northern Japan. Water Sci Technol., 68 (7), 1468-1476.

Kim DJ, Miyahara T, Noike T 1997: Effect of C/N ratio on the bio regeneration of biological activated carbon. Water Sci Technol., 36(12), 239-249.

Knight RL, Payne Jr VWE, Borer RE, Payne Jr RA, Pries JH 2000: Constructed wetlands for livestock wastewater management. Ecol Eng., 15, 41-45.

Langergraber G, Prandtstetten C, Pressl A, Rohrhofer R, Haberl R 2007: Optimization of subsurface vertical flow constructed wetlands for wastewater treatment. Water Sci Technol., 55 (7), 71-78.

Lee CY, Lee CC, Lee FY, Tseng SK, Liao CJ 2004: Performance of subsurface flow constructed wetland taking pretreated swine effluent under heavy loads. Biores Technol., 92, 173-179.

Maltais-Landry G, Chazarenc F, Comeau Y, Troesch S, Brisson J 2007: Effects of artificial aeration, macrophyte species, and loading rate on removal efficiency in constructed wetland mesocosms treating fish farm wastewater. J. Environ. Eng Sci., 6 (4), 409-414.

Megonikal JP, Hines ME, Visscher PT 2004: Anaerobic metabolism: linkage to trace gases and aerobic processes. In: Schlesinger WH, editor. Biogeochemistry, Elsevier-Pergamon, pp.317-424. Oxford, U.K.

Mietto A, Politeo M, Breschigliaro S, Borin M 2015: Temperature influence on nitrogen removal in a hybrid constructed wetland system in Northern Italy. Ecol Eng., 75, 291-302.

Molle P, Liénard A, Boutin C, Merlin G, Iwema A 2005: How to treat raw sewage with constructed wetlands: an overview of the French systems. Water Sci Technol., 51(9), 11-21.

Molle P, Prost-Boucle S, Lienard A 2008: Potential for total nitrogen removal by combining vertical flow and horizontal flow constructed wetlands: A full-scale experiment study. Ecol Eng., 34, 23-29.

Nivala J, Knowles P, Dotro G, Garcı´a J, Wallace S 2012: Clogging in subsurface-flow treatment wetlands: Measurement, modeling and management. Water Res., 46, 1625-1640.

Obarska-Pempkowiak H, Gajewska M 2003: The removal of nitrogen compounds in constructed wetlands in Poland. Pol J Environ Stud., 12(6), 739-746.

Paredes D, Kuschk P, Mbwette TSA, Stange F, Muller RA, Köser H 2007: New aspects of microbial nitrogen transformations in the context of wastewater treatment - a review. Eng Life Sci., 7(1), 13-25

Reeb G, Werckmann M. 2005: First performance data on the use of two pilot-constructed wetlands for highly loaded non- domestic sewage. In: Vymazal, J. (Ed.), Natural and Constructed Wetlands: Nutrients, Metals and Management, pp. 43-51. Backhuys Publishers, Leiden, The Netherlands.

Saeed T, Sun G 2012: A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. J Environ Manage., 112, 429-448.

Savant NK, DeDatta SK 1982: Nitrogen transformations in wetland rice soils. Adv Agron., 35, 241-302.

Shamir E, Thompson TL, Karpiscak MM, Freitas RJ, Zauderer J 2001: Nitrogen accumulation in a constructed wetland for dairy wastewater treatment. JAWRA., 37(2): 315-325.

Sharma PK, Inoue T, Kato K, Ietsugu H, Tomita K, Nagasawa T 2011: Potential of hybrid constructed wetland system in treating milking parlor wastewater under cold climatic conditions in northern Hokkaido, Japan. Water Prac Tech., 6(3): IWA Publishing, http://dx.doi.org/10.2166/wpt.2011.052.

Sharma PK, Inoue T, Kato K, Ietsugu H, Tomita K, Nagasawa T 2013: Seasonal efficiency of a hybrid sub-surface flow constructed wetland system in treating milking parlor wastewater at northern Hokkaido. Ecol Eng., 53, 257-266.

Song X, Li Q, Yan D 2010: Nutrient removal by hybrid subsurface flow constructed wetlands for high concentration ammonia nitrogen wastewater. Procedia Environ Sci., 2, 1461-1468.

Steinmann CR, Weinhart S, Melzer A 2003: A combined system of lagoon and constructed wetland for an effective treatment. Water Res., 37: 2035-2042.

Strous M, Van Gerven E, Kuenen JG, Jetten M 1997: Effects of aerobic and microaerobic conditions on anaerobic ammonium-oxidizing (Anammox) sludge. Appl Environ Microb., 63, 2446-2448.

Tanner C, Sukias J 2003: Linking pond and wetland treatment: Performance of domestic and farm systems in New Zealand. Water Sci Technol., 48, 331-339.

Tanner CC, Kadlec RH 2003: Oxygen flux implications of observed nitrogen removal rates in subsurface-flow treatment wetlands. Water Sci Technol., 48,191-198.

Tanner CC, Nguyen ML, Sukias JPS 2005: Nutrient removal by a constructed wetland treating subsurface drainage from grazed dairy pasture. Agr Ecosyst Environ., 105, 145-162.

Third KA, Olav Sliekers A. Kuenen JG, Jetten MSM 2001: The CANON system (Completely autotrophic nitrogen removal over nitrate) under ammonium limitation: interaction and competition between three groups of bacteria. Syst Appl Microbiol., 24, 588-596.

Tuncsiper B 2007: Removal of nutrient and bacteria in pilot-scale constructed wetlands. J Environ Sci Health, Part A, 42 (8), 1117-1124.

U.S. Environmental Protection Agency, US EPA 1975: Process Design Manual for Nitrogen Control. Office of Technology Transfer, Washington, DC.

Vymazal J 2005: Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Ecol Eng., 25(5), 478-490.

Vymazal J 2007: Removal of nutrients in various types of constructed wetlands. Sci. Total Environ., 380, 48-65.

Vymazal J 2008: Constructed Wetlands for Wastewater Treatment: A Review. Proceedings of Taal2007: The 12th World Lake Conference: 965-980.

Vymazal J 2013: The use of hybrid constructed wetlands for wastewater treatment with special attention to nitrogen removal: A review of a recent development. Water Res., 47, 4795-4811.

Vymazal J, Kröpfelová L 2015: Multistage hybrid constructed wetland for enhanced removal of nitrogen. Ecol Eng., 84, 202-208.

Vymazal J, Kröpfelová L 2008: Is concentration of dissolved oxygen a good indicator of processes in filtration beds of horizontal-flow constructed wetlands? In: Vymazal, J. (Ed.), Wastewater Treatment, Plant Dynamics and Management in Constructed and Natural Wetlands, pp. 311-317. Springer Science Business Media B.V., Netherlands.

Wallace SD, Knight RL 2006: Small-scale Constructed Wetland Treatment Systems: Feasibility, Design Criteria, and O&M Requirements. Water Environment Research Foundation (WERF), Alexandria, Virginia. IWA Publishing, London, UK.

Wang J, Yang N 2004: Partial nitrification under limited dissolved oxygen conditions. Process Biochem., 39 (10), 1223-1229.

Zhang L, Xia X, Zhao Y, Xi B, Yan Y, Guo X, Xiong Y, Zhan J 2011: The ammonium nitrogen oxidation process in horizontal subsurface flow constructed wetlands. Ecol Eng., 37, 1614-1619.

Zhao Y, Zhang H, Xu C, Nie E, Li H, He J, Zheng Z 2011: Efficiency of two-stage combinations of subsurface vertical down-flow and up-flow constructed wetland systems for treating variation in influent C/N ratios of domestic wastewater. Ecol Eng., 37, 1546-1554.

Zhu H, Yan B, Xu Y, Guan J, Liu S 2014: Removal of nitrogen and COD in horizontal subsurface flow constructed wetlands under different influent C/N ratios. Ecol Eng., 63, 58-63.

Acknowledgement

Firstly, I would like to express my greatest gratitude to my supervisor Prof. Takashi Inoue for guiding me in the past three years. It's my great honor to be allowed to study with him, and I learnt so much from him that I think I would remember all my life. I also would like to thank Mr. Kunihiko Kato for his help on the fieldwork and my study. I also would like to thank Dr. Tadao Yamamoto who gave me valuable suggestion during my study. Without their help, I couldn't continue my study in Hokkaido University, Sapporo, Japan.

This study has been carried out at the Laboratory of Land and Water management, Graduate School of Agriculture, Hokkaido, and the lab members do gave me a lot of help during my stay in Japan.

I would like to appreciate my gratitude to my parents for their understanding and support for my study and life in Japan. And I want to thank my friends for their encouraging to my study.

I would like to thank the JASSO that supported me a scholarship (GAKUSYUSYOREIHI) for my study in Sapporo, and to thank Sapporo Nougaku Dousoukai for the financial aid they have provided me to take part in the WETPOL 2015, which was held in York, England. I would also like to express our sincere appreciation to the Ministry of Agriculture, Forestry and Fisheries, Japan for their financial support and the owner of the farms to help us carry out our research. This study was also supported by JSPS KAKENHI Grant Number 26292185.